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A randomized prospective clinical study

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ABBREVIATIONS

APF/V	Apically positioned flap / Vestibuloplasty
BOP	Bleeding on probing
CG	Control group
CYT	Cytokeratin
FGG	Free gingival graft
FU-3	Follow-up 3 months
KT	Keratinized tissue
LH	Lysyl hydroxylase
MMP	Matrix metalloproteinase
PD	Probing depth
PES	Pink esthetic score
PI	Plaque index
SCTG	Subepithelial connective tissue graft
TG	Test group
VG	Volume gain
WES	White esthetic score

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INTRODUCTION

1. INTRODUCTION

The present study is based on the field of soft tissue augmentation around dental implants. Previous investigations (1, 2) have demonstrated that biological processes related with vertical and horizontal bone resorption around the alveolar process happen after tooth extraction. This event may create a volume deficiency in the area causing a non-aesthetic outcome for the patient (3).

Surgical techniques such as alveolar socket preservation (4, 5), guided bone regeneration (6-8) or soft tissue augmentation (9) have arisen to compensate this volume loss in the alveolar process profile. In soft tissue augmentation the majority of these procedures are described as bilaminar techniques using an autogenous subepithelial connective tissue graft obtained from the lateral palate (10). However, emerging evidence (11) seems to point out that autogenous connective tissue from the tuberosity may have better tissue qualities for soft tissue augmentation compared with the palate. Some clinicians advocate the use of tuberosity connective tissue when soft tissue volume is concerned (12). It seems that tuberosity tissue may be a denser tissue with a lower percentage of fat tissue compared with the lateral palate, and therefore could obtain better values in terms of volume gain (11, 13). However there is limited scientific evidence when comparing both areas.

In the present research a clinical and histological comparison between the soft tissue donor areas, palate and tuberosity, has been investigated. Patients with alveolar process deficiency were recruited to perform a soft tissue augmentation procedure using autogenous conective tissue randomly selected from the palate or from the tuberosity. Furthermore, immunohistochemistry evaluating parameters related with collagen turnover has been performed. The histological analysis could give a possible biological explanation for the clinical results obtained.

In the present thesis, the scientific rationale of the study will be discussed, the material and methods used shown and the obtained outcomes explained. Finally,

clinical and histological results will be discussed and compared with the scientific evidence published until now.

BACKGROUND

2. BACKGROUND

2.1 Importance of the quality and quantity of soft tissue

The quality and quantity of soft tissue around implants and teeth has been and is currently a matter of discussion and controversy. Over 35 years ago, Lang & Loe (14) reported better periodontal health in teeth, which yielded at least 2 mm of keratinized gingiva with 1 mm being attached, compared to teeth without 2 mm of keratinized gingiva. Although the latter findings have been challenged by subsequent studies (15-18), a width of 2 mm of keratinized gingiva has been considered clinically desirable to provide an appropriate soft-tissue band around natural teeth, particularly in non-compliant patients with poor oral hygiene (19). However, whether a lack of an adequately keratinized tissue (KT) compromises the maintenance of periodontal health around teeth or dental implants is still in some ways controversial (20-22).

Structural differences in implants (i.e., lack of cementum and periodontal ligament, less vascular supply, parallel orientation of supracrestal connective tissue) and natural teeth (22-25), make implants more susceptible to the development of inflammation and bone loss (26). For these reasons, the prevention of biologic seal breakage around implants is one of the goals in implant maintenance (27, 28).

Researchers and clinicians have questioned whether peri-implant KT is necessary or at least beneficial for peri-implant soft-tissue health, and if the same two mm threshold recommended for natural teeth, also applies to dental implants (20). Studies have been performed to evaluate if the presence of KT is a critical factor influencing the average annual bone loss around dental implants (21, 27, 29). These publications reported that KT appears to be significantly advantageous in the reduction of gingival inflammation and plaque accumulation but not in average annual bone loss (27). However, other investigations suggested that the mean

bone loss was higher for implants with narrow zones of KT (less than 2mm) when using a multivariable linear regression analysis (28).

Wennström et al (30) concluded that neither the width of masticatory mucosa nor the mobility of the border tissue had a significant influence on plaque accumulation and health of the peri-implant mucosa. However, Schrott and co-workers (29) after five years evaluating implants in mandibular fixed prostheses concluded that sites with less KT exhibited higher peri-implant recession. This occurred mainly in the lingual area maybe due to the difficult access to oral hygiene methods. Moreover, a systematic review by Cairo and co-workers (20) reported that a lack of KT does not influence long-term implant survival, but the preservation and/ or the reconstruction of KT around dental implants may facilitate restorative procedures, improve plaque control and render in better aesthetics by reducing the incidence of gingival recession.

Emerging evidence seems to point out a higher risk of peri-implant disease in implants with an absence of KT. In a retrospective cross-sectional study (31) after evaluating 478 implants during 4.43 ± 2.25 years, a statistical association between peri-implantitis and absence of KT was found with an odds ratio of 3.89, 95% CI: 2.34-5.98. Furthermore, recent studies (32), with a longer follow-up (10 years) were performed in patients receiving supportive periodontal therapy. The results indicated that 51,4% of patients with implants without KT needed antibiotic or surgical therapy due to biological complications during this period, whereas this occurred only in 12,7% of patients with implants surrounded by KT. Statistical significant difference between groups were found, with more plaque accumulation and greater soft tissue recession for the non keratinized implants. Also, bleeding on probing (BOP) and mean bone loss were higher for the alveolar mucosa surrounded implants, even though these differences lacked significance.

Some studies have suggested that thick gingival biotype exhibited significantly less facial gingival level change than sites with a thin gingival biotype at 1 year after implant placement (24, 33, 34). In cases of thin periodontal biotype gingival grafting has been advocated to prevent or correct this problem. The resulting tissues seem to be more resistant to recession (33, 34). Furthermore, it is believed

that a thin biotype and non-keratinized gingiva around the natural dentition or around dental implant possess an inherent risk of recession when a surgical/restorative procedures or mechanical trauma is applied (34, 35). In situations where recession occurs at the facial aspect the aesthetics may be compromised particularly if titanium is exposed (24). It is important then to protect soft and hard tissue to achieve stability for both functional and aesthetic aspects (33).

2.2 Importance of soft tissue thickness around dental implants

Soft tissue thickness could be another important point in implant maintenance. Twenty years ago, Berglundh et al (36) performed an animal study to evaluate the importance of soft tissue thickness around dental implants. In a split mouth design, at the time of abutment connection in one side the tissue was made thin (≤2mm), while the other side was non-thinned. After 6 months at sites where mucosa was thin, the wound healing during this period included bone resorption. It was suggested that a certain width of peri-implant mucosa is needed if the main goal is to prevent bone loss. It was concluded that if soft tissue was thin (≤2mm), the wound healing and the formation of biological width will take place involving bone loss.

Furthermore, recent evidence (37-39) seems to indicate that thick tissues could maintain peri-implant bone levels better than thin tissues. In this context, Linkevicious et al performed (38) a study series comparing the influence of thin and thick gingival tissues when implant bone loss is concerned. Tissue thickness was determined at the time of implant placement. After buccal flap was elevated, apico-coronal width of the non-elevated lingual flap was measured with a periodontal probe. If apico-coronal soft tissue thickness was 2mm or less, the tissues were considered as thin, whereas if the thickness was more than 2mm was classified as thick. After implant placement bone levels monitoring was performed by means of radiographic evaluation. Results indicated that thin gingival tissues (≤2mm) had higher bone loss, being at implant mesial aspect -1,65 ± 0,08mm and -1,81 ± 0,06mm at distal aspect, compared with thick gingival tissues (>2mm),

being at mesial area -0,44 ± 0,06mm and -0,47 ± 0,07mm at distal area after 12 months. Another group of the study consisted in thin tissues augmented surgically placing an allogeneic membrane. In this group the bone loss values decreased up to -0,31 ± 0,05mm mesially and -0,34 ± 0,05mm distally, preserving more bone than the thin group. It was concluded that implants in naturally thick gingival tissues experienced less bone remodelling than thin gingival tissues. Furthermore, thickening of thin tissues with an allogeneic membrane may reduce bone resorption at 12 months. These outcomes have been discussed in a subsequent systematic review (40) where after analysing the available data, it was concluded that there is insufficient long-term evidence to confirm that augmented soft tissues can be maintained over time and able to prevent peri-implant bone loss.

In the anterior areas, it seems more clear that an absence of a proper soft tissue around dental implants may cause deficient aesthetic outcomes (3). A previous study confirmed that four parameters, among others, had a greater influence in aesthetic evaluation: crown form, contact-point position, color, and topography of surrounding soft tissues (41). It seems that obtaining a favourable soft tissue contours around the clinical implant crown is essential to achieve an esthetic result (42). This have to include a healthy soft tissue appearance with a correct height, volume, color and contour; which must be in harmony with the adjacent natural teeth (43).

It is also shown in the literature, that biological processes related with bone resorption occurs after tooth extraction, also in cases where an immediate implant is placed (1, 2). This event may cause an alveolar process volume deficiency leading to a non-aesthetic outcome. In another study, it was demonstrated (3) that unfavourable aesthetics results caused by this phenomenon occurred around 20% of cases when using immediate implants.

Different methods have been used to evaluate esthetic results around dental implants. Jemt et al (44) evaluate the esthetic result regarding the amount of papilla present. Belser and co-workers (45) created the White Esthetic Score

(WES) to evaluate the implant supported crown itself and the and Pink Esthetic Score (PES) to evaluate the soft tissue surrounding the crown. Probably these are the most used classification systems. WES is based on the following parameters: general tooth form, outline and volume of the clinical crown, color, surface texture and translucency-characterization. While the following five parameters are evaluate at PES: mesial papilla, distal papilla, curvature of the facial mucosa, level of the facial mucosa, and root convexity/soft tissue color and texture at the facial aspect of the implant site. A score of 2, 1, or 0 is assigned to each parameter. The classification for papillary tissue is: complete presence (score 2), incomplete presence, (score 1), or absence (score 0) of papilla. The curvature of the facial soft tissue profile (line of emergence of the implant restoration) is assessed as being identical (score 2), slightly different (score 1), or markedly different (score 0) compared to the natural control tooth. The level of the facial mucosa is scored by comparison to the contralateral tooth in terms of an identical vertical level (score 2), a slight (≤1 mm) difference (score 1), or a major (≥1 mm) difference (score 0). PES combines three additional specific soft tissue evaluations as one variable: the presence, partial presence, or absence of a convex profile (in concordance to a root eminence) on the facial aspect, as well as the related mucosal color and surface texture. At PES more factors than the papilla are evaluated. This could be a better classification as it is demonstrated that soft tissue profile and topography of surrounding soft tissues are also directly involved in aesthetics (3, 41).

A study (46) was performed to evaluate whether the use of soft tissue augmentation procedures around implants could improve the aesthetics outcomes. This was a randomized controlled split-mouth design conducted in patients that required at least one implant bilaterally. After implant placement, one side was augmented by means of a subepithelial connective tissue graft (SCTG) from the palate, while the other side was non-grafted. Then at 1 year, a blinded examiner evaluated the PES on both sides. Results showed statistically significant better values for the augmented areas. Also patients were more satisfied with the augmented sites due to a better aesthetic outcome.

From the prosthetic point of view, gingival thickness seems to be also a critical aspect. An in vitro study (47) concluded that a minimum of 2mm gingival thickness around the abutment is needed to avoid gingival discoloration from the abutment and to achieve a proper aesthetic outcome. These results were confirmed in two in vivo studies (48, 49) where it was concluded that in areas with gingival thickness of 2mm or less, all ceramic restorations obtained the minimum color change in gingiva. Sala et al (50) compared the effect on gingival color when placing different abutments. They concluded that the natural gingival color was not reproduced with any abutment at gingival thicknesses < 2 mm. It seems that the worst color match was observed when using titanium abutments, whereas the best color match was obtained with fluorescent zirconia. However, at areas with gingival thickness > 2 mm, no differences were detected among abutments. Emerging evidence seems to point out that gold or zirconia abutments may be the best solution to avoid gingival discoloration when gingival thickness is < 2mm (51, 52). It is recommended that in anterior areas with thin peri-implant soft tissues, titanium abutments should be avoided because of the low colorimetric performance (52).

2.3 Surgical approaches for soft tissue augmentation

Understanding the importance of the surrounding soft tissue in dental implants has lead some clinicians to develop surgical approaches to augment this tissue (53, 54). Different techniques have been compared, in pursuit of which could be the gold standard to achieve more soft tissue volume. In this context, Studer and co-workers (55) recruited 24 patients with a localized alveolar ridge defect and compared SCTG versus Free Gingival Graft (FGG). In order to analyse volumetric changes a projection Moiré system was used. It was reported that SCTG obtained a greater volume gain (VG) with significant statistical differences compared with the control group (FGG) at 1 and 3.5 months. Both techniques have been also compared in terms of root coverage obtaining better values in the SCTG. At this investigation (56) both procedures were compared obtaining root coverage of 53,2 ± 21,5% when FGG was used, while the group of SCTG achieved 85,2 ± 17,9%. Using FGG only 8,8% of cases obtained a complete root coverage, whereas in

patients who received SCTG this represented a 48,6%. Other studies evaluated both techniques in terms of patient centered outcomes concluding that SCTG was painless compared with the FGG when evaluating the donor site (57).

However, the FGG procedure is still being the gold standard when KT gain is the goal. In this aspect (58) an apically positioned flap/vestibuloplasty (APF/V) plus autogenous tissue seems to be the treatment of choice. This procedure was compared with scaling and root planning and with untreated controls obtaining better values in KT gain. Also, the addition of autogenous tissue to an APF/V improved the outcome compared with an APF/V alone. A recent systematic review (59) amounted the mean gain of KT after applying different surgical techniques. The mean KT gain after evaluating all techniques ranged from 1.2 ± 0.8 to 2.6 ± 0.5 mm. Three APF/V techniques combined with FGG, SCTG or xenogeneic graft material achieved similar peri-implant KT gain. When implant dehiscence coverage was the main goal, both split thickness flap with SCTG and coronally advanced flap with SCTG were equivalent in recession coverage around implants.

Following a tooth extraction, biological processes related with bone resorption occurs. Different surgical approaches such as alveolar socket preservation (4, 5), guided bone regeneration (6-8), soft tissue augmentation (60) or a combination (61) have been used to compensate this phenomenon. Eghbali and co-workers (60) evaluated the use of SCTG using an ultrasonic device to check the soft tissue gain. The soft tissue augmentation procedure was performed after a provisional crown was placed and 3 months following implant placement. A soft tissue thickness increase of 0,83mm was obtained at 9 months after performing a SCTG from palate. It was concluded that SCTG was able to thicken the peri-implant mucosa with stable results at 9 months. Also soft tissue augmentation has been used with immediate implants trying to compensate the volume loss due to the alveolar resorption. Grunder et al (62) evaluate the crestal ridge width changes comparing 12 patients who received immediate implants with SCTG at the same time versus 12 patients who were treated with immediate implants only. Results at 6 months showed that grafted immediate implants gain 0,3mm, whereas non-grafted immediate implants had a loss of volume of 1,0mm. It was concluded that

using SCTG at the time of immediate implant placement is an effective procedure to compensate the expected volume loss in the alveolar process.

Also soft tissue augmentation procedures may be performed at second stage implant surgeries. Speroni (63) reported a soft tissue thickness gain around implants after performing different surgical approaches (free connective tissue graft and SCTG either from tuberosity or from palate). The results of 40 patients showed a mean gain of 1,4 mm in tissue thickness at 36 months after surgery, indicating that soft tissue thickness may be increased at a second stage surgery. In cases where at baseline the mucosa was thinner, a higher increase was observed.

Systematic reviews have been performed in this field. Thoma et al (58) reported, after evaluating the available literature, the superiority of SCTG among other surgical techniques when soft tissue volume augmentation is the goal. It is important to underline as a limitation that in the volume augmentation evaluation only one study could be used. Even though, this finding was in agreement with other studies (55, 64).

The majority of these procedures are described as a bilaminar techniques obtaining a SCTG from the premolar palatine area (65). However, some surgeons preferred to harvest the SCTG from the tuberosity area (12) showing better tissue qualities and less patient discomfort when compared with the palate.

Xenogeneic materials have been tested for soft tissue augmentation, in terms of gain of KT. Lorenzo et al (66) compared a xenogeneic collagen matrix versus a free connective tissue graft around dental implants. No statistically significant differences were found, obtaining a mean increase in KT of 2,3mm and 2,33mm, respectively at 6 months. Even though without statistical significance, patients treated with the collagen matrix referred less pain and needed less medication compared with the free connective tissue group. A similar trend was observed in a study (67) where the VG was the main goal. In this randomized controlled clinical trial, a collagen matrix was compared with a SCTG from palate. After 3 months similar values were obtained for both groups with non-statistically significant

differences. However, the non-inferiority of collagen matrix could not be demonstrated, since the study design was underpowered because the variability used in the sample calculation was too small. In terms of patient centered outcomes, no statistically significant differences were found, but collagen matrix group needed less medication. The study conducted by Schallhorn et al (68) is in agreement with these previous studies. A xenogeneic collagen membrane was placed at buccal aspect of the implants of thirty patients and thickness/KT increase was evaluated at 6 months. Results showed that the xenogeneic membrane was able to increase both soft tissue thickness and KT with statistically significant differences between 6 months and baseline.

Using soft tissue augmentation techniques at the time of implant placement or abutment connection can improve aesthetic outcomes and stability of peri-implant soft tissues (46, 69). However, there is only weak evidence and it is still unclear which is the best soft tissue augmentation procedure (70). Currently, the long-term success of these surgical procedures around dental implants is still unknown (71).

2.4 Donor areas for autogenous subepithelial connective tissue graft

In soft tissue augmentation procedures autogenous connective tissue can be obtained from the palate, maxillary tuberosity, or edentulous ridges (72-74). The palate is the most common donor site, however, its dimensions can affect the amount of connective tissue that can be harvested (73). Care is taken to avoid the greater and lesser palatine nerves and vessels (75-76). It has been reported that the mean height of the secure area to harvested the connective graft is 14.9 +/– 2.9mm in men and 12.7 +/– 2.4mm in women (77) .

There appears to be certain controversy about the palate thickness. While Barriviera and co-workers suggested that the thickest area was in the second molar with 3,15mm (78), Song et al using a CT technique reported the second premolar region as the thickest with 3,81 mm (79). However both studies showed a tendency of an increase of thickness from the canine to the premolars, a thinner

part in the first molar area and a new increase at the second molar area; being the areas of premolars and second molar the thickest. These results are in contrast with the ones published by Müller and co-workers using a ultrasonic measuring device SDM (80, 81), where it was showed that tuberosity area was the thickest with more than 4mm, while at the premolar area the thickness was an average of 2.9mm about 6-10mm distant from the respective gingival margin of the tooth. These outcomes were in accordance with an investigation (82) performed in 20 cadavers, where a similar thickness was observed in the tuberosity area with an average between 2,5 and 4mm.

The histologic composition of the palate connective tissue has been studied (11, 83). It is composed basically of three layers: the epithelium, the connective tissue with the lamina propria and the submucosa. In a previous study (84), it was reported a mean epithelial thickness of 430,63 microns, with a range of 113-823 microns. The epithelium is orthokeratinized and underneath there is the connective tissue (lamina propria). The major component of the connective tissue are the collagen fibers. These fibers represent around 60% of the connective tissue and are surrounded by the extracellular matrix, which is produced mostly by the fibroblasts. The extracellular matrix is the responsible for the maintenance of the connective tissue. The lamina propria has two portions, being the papillary portion the most superficial and the reticular portion the deepest. The papillary portion shows vertical projections to the overlying epithelium (rete pegs) and the reticular portion consists basically of thick and dense reticular fibers. Between this layer and up to the alveolar bone there are numerous glands, nerves and fat tissue, which formed the submucosa. It is described that in the anterior palate the submucosa is characterized by rather fat tissue while in the posterior areas is mostly formed by glandular tissue. It is also smaller in the posterior than in the anterior area of the palate. However, a high interindividual variation in lamina propria and submucosa proportions has been found in histological human studies (85, 86). Samples were obtained by means of SCTG, and after histologic examination some grafts consisted mainly in lamina propria, while others presented mostly submucosa (85).

Bertl and colleagues (86) obtained palate samples from 10 fresh human cadavers, and also a high variability was observed. The hypothesis of this study was to find out whether the harvesting technique and the area of the palate (anterior – posterior and marginal - apical) could influence the histological composition of the graft. Tissue samples were harvested in the anterior and posterior palate and in areas close to (marginal) and distant from (apical) the gingival margin. Also two harvesting techniques were compared: split flap and de-epitelization of a free gingival graft. Statistical significant differences were found regarding harvesting technique, obtaining higher proportions of fibrous connective tissue and significantly lower proportions of fat/glandular tissue when utilizing the deepitelization technique compared with the split flap technique, irrespective of donor site. Moreover, no statistical significant differences were found regarding the harvesting area, but more fibrous connective tissue was encountered for both techniques in the anterior and marginal area of palate.

In accordance with the previous study, Sun et al (87) found more lamina propria thickness in the marginal area of 34 palatal samples compared with the area close to the midpalatal suture. The lamina propria thickness at the area close to the gingival margin was 2.06 ± 0.70, whereas at 8mm from gingival margin this layer had a thickness of 1.28 ± 0.46 mm.

In a classical study, Ouhayoun et al (88) harvested a thick palatal FGG, which was split into two thinner grafts, a superficial epithelium-connective tissue graft and a deep connective tissue graft. Both grafts were transplanted into recipient mucosal beds lacking keratinized gingiva and at 3 months postoperatively, punch biopsies were analysed. The analysis consisted in descriptive histology, immunofluorescence with different anticytokeratin antibodies and biochemical techniques with non-equilibrium two-dimensional gel electrophoresis. Results demonstrated that sites receiving the superficial graft showed histologic and biochemical properties of keratinized mucosa. Otherwise, sites grafted with deep connective tissue mostly showed characteristics of non-keratinized gingiva.

After analysing these previous investigations it may be speculated that a high interindividual variation in lamina propria and fat/glandular tissue contain exists,

and also that depending on the proximity to the epithelium the harvested graft may have different composition and behaviour when grafted.

The maxillary tuberosity, in contrast, is reported to have less variable and thicker soft tissue than the hard palate and may be a suitable donor source for connective tissue graft (89, 90). Literature reports that the thickest grafts can be obtained in the tuberosity region (i.e., 5mm)(91).

Although not all patients have a large tuberosity, obtaining connective tissue from this area could have advantages over the palatal mucosa. Recent studies (11, 12) have demonstrated that SCTG from the tuberosity is a very dense and collagen-rich tissue that seems to contain more collagen and less fat and glandular tissue compared to the anterior lateral palate.

Shrinkage of the connective tissue commonly occurs after harvesting it from the palatal mucosa (92). However, the contraction of the connective tissue obtained from the tuberosity area seems to be less (11). The phenomenon of creeping attachment (reported before using free gingival grafting and connective tissue grafting with partial-thickness double pedicle) is observed also with SCTG harvested from the tuberosity area (90). Furthermore in some cases an additional gingivoplasty is required (13, 89).

Harvesting the graft from the tuberosity, in some cases also allows to combine two procedures: reduction of distal periodontal pockets (distal wedge) and augmentation of soft tissues (90).

Tuberosity autogenous connective tissue has been used for root coverage treatment. The results obtained appear to be similar of the ones obtained with a palate tissue graft. While a systematic review (93) has shown a success rate of root coverage of 64,7-95,6% with a predictability of 83,3% of complete root coverage using the graft from the palate, Aroca and co-workers (94) reported a 82% of root coverage in Miller Class III demanding cases using tuberosity connective tissue graft with a tunnel modified technique. Similar results were obtained using enamel matrix derivative together or not.

Other studies have shown better results. Zucchelli et al achieved a 97% of the root surface coverage and 88% complete root coverage when using connective tissue graft from the palate to treat gingival recessions (95). However, Hirsch (72) obtained a 95% root coverage with 84,1% predictability using the tuberosity connective tissue as a donor site. Furthermore, Jung et al concluded that using the tuberosity to harvest the connective tissue graft is an easy method with a highly predictable prognosis (89).

In cases of reconstruction of the papilla some clinicians preferred to use connective tissue graft from the tuberosity affirming, again, that its connective tissue tends to be more dense, more fibrous and thicker (96, 97).

Even thought, SCTG from tuberosity has been used for several surgical procedures, there is limited scientific evidence comparing SCTG from palate versus tuberosity. Dellavia (13) has compared clinically and histologically both donor areas for ridge augmentation, obtaining better values in the tuberosity group 6,4mm versus 4,7mm compared with palate group when soft tissue volume is concerned.

2.5 Immunohistochemistry related with soft tissue modulation

When soft tissue VG is concerned, it may be speculated that a more dense and fibrous tissue with a higher content of collagen could obtain more VG as a consequence of less postoperative contraction (11). Therefore, mechanisms related with collagen turnover may have an interest in the field of soft tissue augmentation.

It is known from previous studies that matrix metalloproteinases are related with collagen degradation in the periodontal environment (98). Ejeil and co-workers (99) compared the amount of collagen fibers and matrix metalloproteinases in three groups of patients with different grade of gingival inflammation (healthy, mild, moderate, severe). The area fraction occupied by collagen fibers resulted for healthy gingiva 53 ± 11%, compared with the 35,25 ± 8% for the severe gingival

inflammation. Results regarding matrix metalloproteinases indicated that there was an inverse correlation between collagen fibers and matrix metalloproteinases, especially matrix metalloproteinase (MMP)-2, MMP-9, MMP-1 and MMP-13. Also as the gingival inflammation was higher, the more decrease of collagen fibers was observed.

Otherwise, in cases of gingival idiopathic overgrowth an excessive gingival accumulation is suffered. It has been hypothesized that the cause of this gingival accumulation may be an alteration of the collagen regulation pathways. This may lead to a deposition of extracellular matrix components such as collagen (100). Histologic comparison (101) between gingival overgrowth patients and healthy ones indicated that in the epithelium of the gingival overgrowth patients no acanthosis and more collagen fiber bundles were found. Also, the molecular analysis showed higher values of collagen type I, MMP-1 and lysyl hydroxylase (LH) 2b for gingival idiopathic overgrowth patients, whereas collagen type III was almost the same between samples. The mean difference between groups remained in LH 2b values, which is related with the collagen cross-linking.

In the biosynthesis of collagen, cross-linking is the final step. There are two ways to perform this step, the allysine route and the hydroxyallysine route. In the first route, a lysine residue within the telopeptide is converted by lysyl oxidase into the aldehyde allysine. In the second route, a hydroxylysine residue within the telopeptide is converted into the aldehyde hydroxyallysine (102). It have been observed in fibrotic processes an increase formation of hydroxyallysine cross-links, whereas the allysine route decrease (103). These suggest that these type of cross-links may be more difficult to degrade, contributing to the collagen accumulation (102).

LH 2b has been described as a telopeptide lysyl hydroxylase. It is responsible for the overhydroxylation of the collagen telopeptides and then to the formation of pyridinolines cross-links. Pyridinoline cross-links are derived from hydroxylated lysine residues located within the collagen telopeptides (102). The resulted collagen which contain pyridinoline cross-links contribute to the unwanted collagen

accumulation, which is found in fibrotic processes (104). This leads to an hypothesis that the collagen accumulation may be due to a posttranslational mechanisms, that increase collagen cross-links which makes collagen fibers less susceptible to degradation by matrix metalloproteinase (101).

Similar clinical and molecular results were obtained in patients after a SCTG from tuberosity was performed. Dellavia et al (13) compared the soft tissue gain using SCTG from palate or tuberosity for ridge augmentation. It was concluded that patients receiving a tuberosity SCTG tended to an excessive gingival accumulation, which had to be surgically removed. Also histological differences observed seems to indicate that collagen fibers of the tuberosity can be more susceptible to cross-linking than the palate ones, and therefore less prone to degradation. It is important to underline that no statistically significant differences were found in the histological outcomes, but a non-statistically significant increase of LH 2b / Collagen-I mRNA in fibroblasts from the tuberosity characterized by a hyperplastic response was found. This means that tuberosity collagen fibers could be more susceptible to cross-linking and therefore less susceptible to degradation by MMPs, leading to its excessive deposition. It is shown in the literature that MMP-1 cleaves the collagen triple helical region, allowing its further degradation by MMP-2 which is a less specific proteinase (105). In terms of MMP values no statistical significant differences between palate and tuberosity were found in Dellavia study (13), but MMP-1 activity was downregulated in tuberosity. This event together with the non-statistically significant increase levels of LH 2b /Collagen-I mRNA ratio could explain a possible mechanism responsible for collagen accumulation.

Nevertheless, there is limited scientific evidence in collagen behaviour in soft tissue augmentation. Then, it may be speculated that donor tissues with less MMP and higher LH 2b values could maintain a higher amount of collagen fibers and therefore achieve more stable results. Evaluating some of this parameters related to collagen regulation may be interesting in soft tissue augmentation procedures.

2.6 Scientific rationale

Until now to the best of our knowledge, there are no studies comparing clinically and histologically SCTG from palate and tuberosity when soft tissue volume gain around dental implants is concerned. For this reason the present study aims to compare both areas around implants.

The results of the present study will bring more knowledge to clinicians whether to use palate or tuberosity tissue as a donor tissue area when soft tissue volume gain around dental implants is concerned. The use of optical scan images will give accurate data in terms of volume changes in the three-dimensions.

Also with the results of the histochemistry parameters evaluated (LH 2b, MMP 1-2, and monoclonal antibody against CYT 4-10-13), we may understand the behaviour of these two different tissues when grafted. Some of the parameters evaluated are related with the cross-linking and the breakdown of the collagen fibers, which may be a key factor in soft tissue VG.

HYPHOTESIS

3. HYPOTHESIS

First hypothesis:

Ha0: SCTG from the tuberosity provides equal soft tissue VG around dental implants when compared to palate.

Ha1: SCTG from the tuberosity provides more soft tissue VG around dental implants when compared to palate.

Second hypothesis:

Hb0: SCTG from the tuberosity and palate have similar histologic composition and similar levels of LH 2b, MMP 1-2 and CYT 4-10-13.

Hb1: SCTG from the tuberosity and palate have different histologic composition and different levels of LH 2b, MMP 1-2 and CYT 4-10-13.

OBJECTIVES

4. OBJECTIVES

The main goal of this study is to evaluate and to compare the soft tissue VG around dental implants after grafting a SCTG from two different areas (palate versus tuberosity).

The secondary goal of this study is to evaluate differences in the histologic composition between both tissues using morphologic methods to observe tissue structure and immunohistochemistry to analyse differences in levels of LH 2b, MMP 1, MMP 2 and CYT 4, 10, 13. Also, the secondary objective is to compare changes between groups in the following clinical parameters: plaque index, bleeding on probing, probing depth and width of keratinized tissue.

MATERIAL AND METHODS

5. MATERIAL AND METHODS

5.1 Study design

The present study was designed as a randomized controlled clinical trial with a parallel design in order to evaluate if a significant difference in terms of soft tissue augmentation exists depending on the donor area. Both the study design and informed consent were approved by the Comitè Ètic d'Investigació Clínica (CEIC) with a code PER-ECL-2011-10-NF.

Following the study design and the CONSORT guidelines, the timing was developed as follows (Figure 1):

- Power calculation.
- Patient recruitment and selection.
- Confirmation of inclusion and exclusion criteria.
- Informed consent acquisition.
- Initial therapy.
- Initial data collection and surgical procedure.
- Postoperative care and follow-up.
- Data collection at 3 months.

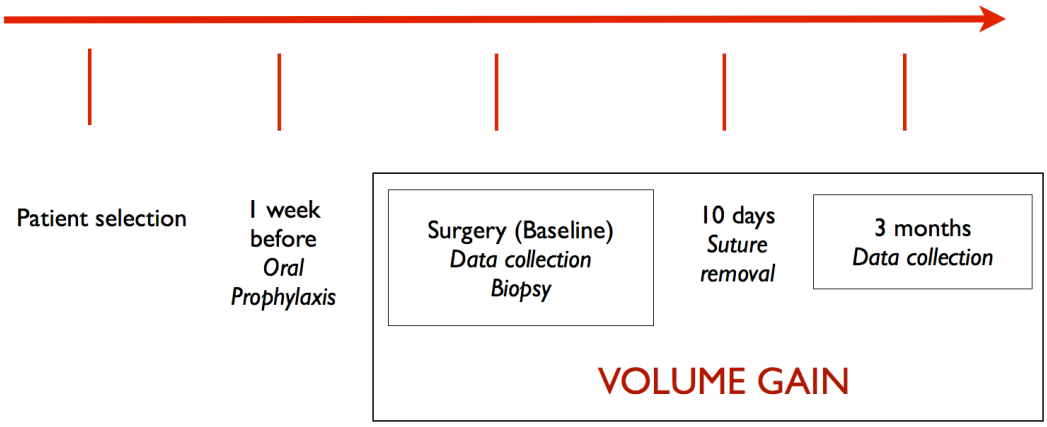

Figure 1. Treatment sequence and time points of evaluation.

5.2 Power calculation

The power calculation was done based on a recent study (13) that compares SCTG from palate and tuberosity around teeth, where a mean difference between both groups was 1,2mm and the standard deviation 1,1mm.

Therefore, for the power calculation, standard deviation was 1,1 in a level of 95 (alpha risk 0,05), power of 80 (beta risk 0,2), expected difference to be detected 1,2 and a dropout rate of 15%. This power calculation resulted in 16 patients per group.

5.3 Sample selection

So 32 patients with unrestored single-tooth implants with volume deficiency were included in order to analyse and compare the obtained data (presurgical baseline compared to postsurgical parameters at 3 months). A maximum of 2 implants per patient were accepted. Two groups were created, control group (CG) for patients receiving palate SCTG, and test group (TG) for the ones receiving tuberosity SCTG. One person (E.R.) performed the recruitment, from May 2012 to November 2016, until the sample was completed.

Inclusion criteria:

- Patient must be ≥18 years old and able to understand the nature of the proposed surgery and to sign the informed consent.
- Patients must have a healthy periodontium.
- Single tooth implants located between two natural teeth.
- All implants locations with a need of a soft tissue volume augmentation as determined by a concavity that was present in the edentulous area or tissues that were thinner than 2mm.
- Palatal tissue must have ≥2mm of thickness in the premolar area and the mesio-distal dimensions of the tuberosity >12mm.
- Full mouth plaque and bleeding scores <20%.

Exclusion criteria:

- Previous soft tissue augmentation in the area.
- Heavy Smokers (> 10 cigarettes per day).
- Local or systemic conditions that would interfere with routine periodontal therapy.
- Allergy to Non-Steroidal Anti-Inflammatory Drugs.
- Patients taking medications that cause gingival enlargement or the presence of gingival idiopathic overgrowth.

5.4 Randomization

Allocation to either treatment was performed according to a computer-based block randomization table (Microsoft Office Excel, Microsoft, Redmond, USA). From the table, black envelopes with group allocation were generated and assigned to each case. This was performed by the statistician (C.E.) who did not participate in the interventions to the patients.

Baseline data and intraoral optical scan were collected. Intraoperative and after the recipient site was prepared, the envelope was opened and group allocation communicated to the surgeon. Blinding allocation was maintained until the recipient site was prepared and after the initial data was recorded.

5.5 Outcome measurements

5.5.1 Primary outcome variable - Soft tissue VG

An intraoral optical scanner (Lava Chairside Oral Scanner C.O.S., 3M ESPE, Seefeld, Germany) was used to obtain an STL file in baseline and 3 months post surgery (FU-3). The optical imaging impression included the implant and at least two adjacent teeth (mesial and distal).

5.5.1.1 STL image matching and volume analysis

STL files obtained from the intraoral optical scan were uploaded to an image analysis software (Geomagic Qualify 12, 3D Systems, Rock Hill, USA). Superimpositions of baseline STL files and FU-3 STL files were obtained for each patient by a blinded examiner (O.G.M.) to evaluate volumetric changes. To achieve the best alignment in the superimposition was mandatory to have a reference and fixed point, which was the healing abutment.

To match the STL images, similar vestibular and buccal surfaces of mesial and distal teeth to the implant were selected. A superimposition was achieved based on the best match of these selections using 300 randomly selected points to get an initial orientation. A further fine adjustments based in 1500 points were performed to achieve the final alignment.

A vestibular area of interest at the implant site was defined. Then, the volumetric changes were calculated by the dedicated software based on linear measurements. Before starting with the measurements, the baseline model was set as reference, while the FU-3 model was set as test.

5.5.1.2 Image analysis

Linear measurements: For each superimposition, labio-palatal sections were obtained perpendicular to the axis of the healing abutment. Then, the linear distance between the baseline and FU-3 soft tissue profile was measured in each mm, from 1 to 7mm, in an apical direction from the healing abutment (Figure 2).

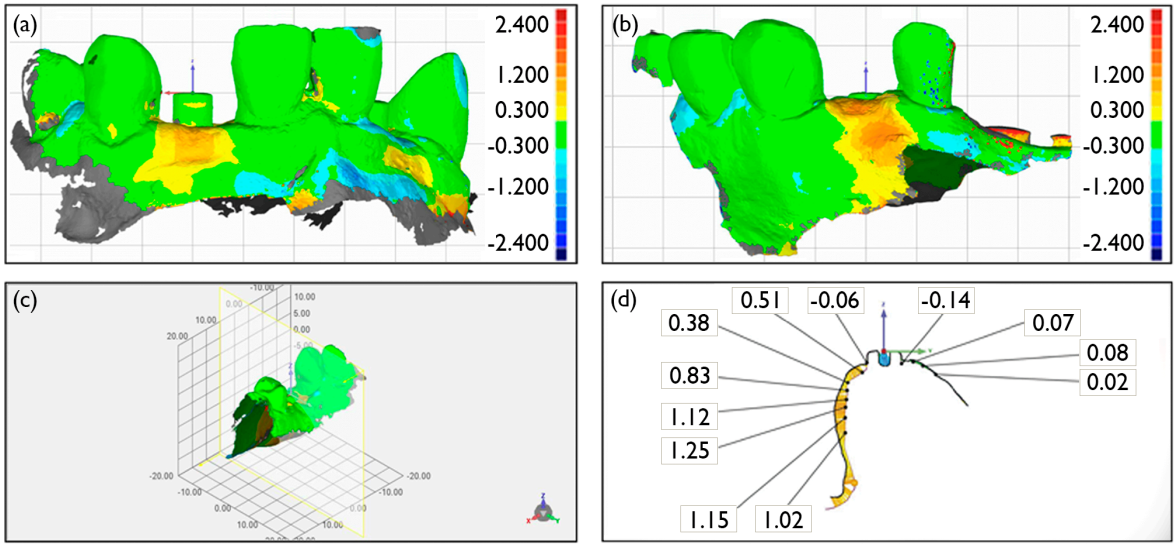

Figure 2. Example of STL image matching and linear measurements. (a) Superimposition of STL files from baseline and FU-3 of a CG patient. No changes occurred in the green areas. The scale indicates different volumetric changes. It can be observed that major changes occurred at the area where SCTG was performed. (b) Superimposition of STL files from baseline and FU-3 of a TG patient. (c) Labio-palatal sections perpendicular to the alveolar bone were obtained crossing in the middle of the healing abutment. (d) The linear distance between baseline and FU-3 STL profiles was measured.

5.5.2 Secondary outcome variable – Histology and clinical parameters

5.5.2.1 Histology

20 histologic evaluations (9 control group, 11 test group) were performed in order to compare palatal and tuberosity tissue samples. Two examiners (E.M., I.S.M) performed all the laboratory procedures. All pieces from human palate (average size 1x1x5 mm) were fixed with buffered 4% formaldehyde (Applichem Panreac; Barcelona, Spain) over night. After fixation, samples were dehydrated with increasingly graded alcohols using a Tissue Processor MTP (Slee Medical; Mainz, Germany) as follows:

- Ethanol 70º……20 minutes
- Ethanol 80º…….20 minutes
- Ethanol 90º……20 minutes
- Ethanol 100º…….30 minutes
- Ethanol 100º……30 minutes
- 1-Butanol……45 minutes
- 1-Butanol……1 hour
- Paraffin…… 1hour 30 minutes
- Paraffin…… 3 hours

Subsequently, standard paraffin embedding was performed, and 3 µm thick sections were obtained with a Rotatory Microtome CUT4062 (Slee Medical). Some of these sections were selected for the posterior analysis. In the selected slides, paraffin was removed and tissues were rehydrated before the staining with haematoxylin-eosin or the immunohistochemistry process, as follows:

- Xylene (2 steps)……15 minutes
- Ethanol 100º……5 minutes
- Ethanol 90º……5 minutes
- Ethanol 70º……5 minutes
- Distilled water…… 5 minutes at least

Haematoxylin-eosin staining

After rehydrating, sections were stayed 7 minutes in Mayer's Haematoxylin (Sigma-Aldrich Inc.; St. Louis, EEUU), and were washed 5 minutes with tap and distilled water. Afterwards, samples were stayed 5 seconds in eosin-floxin and washed again with tap and distilled water in order to eliminate the staining excess. Eosin-floxin was made with Eosin Yellowish solution 1% (Applichem Panreac) and

Floxin B (Applichem Panreac) diluted at 1% in distilled water. These are the stock solutions necessary to obtain the final eosin-floxin:

Product		Final concentration
Stock Eosin	……	10%
Stock Floxin	……	1%
Ethanol 96º	……	78%
Acetic acid glacial	……	0.4%

After removing the eosin, samples were finally dehydrated as follows:

- Ethanol 70º……5 minutes
- Ethanol 90º……5 minutes
- Ethanol 96º……5 minutes
- Ethanol 100º……5 minutes

At the end, all sections were washed 3 times with xylene and mounted with a mounting medium *Vitro-Clud®* (R. Langenbrinck Labor- Medizintechnik Inh.Sibylle; Emmendingen, Germany) for optical evaluation. Histomorphometric analysis to evaluate tissue structure was performed.

Immunohistochemistry

In the immunohistochemistry process the first step after the rehydration of the sections, was to use Ethylenediaminetetraacetic acid (EDTA; Applichem Panreac) for antigen retrieval. EDTA 1mM (pH=8) was preheated in a microwave before the sections were incubated 40 minutes in oven at 100ºC. After cooling the EDTA, slides were washed 3 times (5 minutes each) with wash buffer. In this analysis TRIS was used as a buffer (Table 1).

Solution		Final dilution
Tris for buffer solutions [1]	……	0.6%
Sodium chloride	……	0.88%
Clorhydric acid (1N)	……	4%
Triton ® 100X [1]	……	10%
Adjust pH=7.4-7.5		
Replenish with distilled H_2O		

Table 1. Immunohistochemistry process. Preparation of the TRIS buffer, which was used for washing sections during the immunohistochemistry process.

1) Applichem Panreac.

Then the following incubations were performed: blocking (endogenous peroxidase), primary and secondary antibodies. All incubations were carried out in dark and humidity chamber.

A peroxidase-catalysed visualization method was used. For these reason, the second step in the immunohistochemistry process consisted in block the endogenous peroxidase with blocking. The blocking reagent was included in the "Master Polymer Plus Detection System (Peroxidase)" kit (Master Diagnóstica; Granada, Spain). This step was performed in darkness, at room temperature for 10 minutes. Then, slides were washed 3 times in wash buffer.

Following, all primary antibodies were prepared by dilution with TRIS buffer solution (Table 2).

Primary antibody	Description	Dilution
Matrix metalloproteinase 1(MMP 1)[a]	Mouse monoclonal antibodies	(1:50)
Matrix metalloproteinase 2 (MMP 2)[a]		(1:50)
Cytokeratin 4 (CYT 4)[a]		(1:50)
Cytokeratin 10 (CYT 10)[a]		(1:50)
Cytokeratin 13 (CYT 13)[a]		(1:50)
Lysyl hydroxylase-2b (LH 2b)[b]		(1:150)

Table 2. Antibodies used for immunohistochemistry analysis. Primary antibodies used for each parameter evaluated and its dilution. [a)] Santa Cruz Biotechnology Inc.; California, USA [b)] Novus Biologicals, LLC; Abingdon, UK.

After the blocking reagent was drained from the sections, the prepared diluted primary antibody was added. The slides with primary antibody were incubated over night at 4ºC and washed with TRIS. Then, according to the "Master Polymer Plus Detection System (Peroxidase)" kit (Master Diagnóstica; Granada, Spain) instructions, a secondary antibody was used to detect the union of the primary antibodies. This secondary antibody is based on micro-polymers, suitable for mouse and rabbit monoclonal and polyclonal primary antibodies.

Finally a visualization/development solution, DAB (3,3'-diaminobenzidine), contained in the same kit was used. When the development was complete, slides were washed in tap water during 5 minutes. Then, nuclei were counterstained using Mayer's Haematoxylin during 5 minutes. At the end, tissues were dehydrated and mounted for optical examination as described in the previous section: "Haematoxylin-eosin stained".

In order to obtain control sections, some slides did not follow the full procedure and were incubated with TRIS instead of primary and secondary antibody.

Analysis of the processed samples

After all samples were mounted on coverslips, there were analysed using a Leica DMR microscope (Leica Geosystems AG, St. Gallen, Switzerland) and photographed with a Leica DFC 320 digital camera. The main tissue layers (epithelium, connective tissue and submucosa) were analysed and compared between groups. Also, immunohistochemistry results were analysed.

5.5.2.2 Clinical parameters

The following clinical periodontal parameters were assessed by 3 blinded, experimented and calibrated examiners (G.S., B.P., E.R.) from the Periodontology department. Measurements were performed at baseline and FU-3. Parameters evaluated were:

-Plaque index (PI): Each tooth was divided in 4 surfaces: buccal, lingual, mesial, distal. After the application of erythrosine, O'Leary plaque index was registered.

-Bleeding on probing (BOP): Each tooth was divided in 4 surfaces: buccal, lingual, mesial, distal. After a gentle probing if the gingiva bleeds was positive.

-Probing depth (PD): at the implant area (implant and both teeth next to it). Distance from the gingival margin to the bottom of the gingival sulcus using a periodontal probe (UNC15). Measuring in 6 points: mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual.

-Width of KT: at the implant area (implant and both teeth next to it). It was measured in the medial buccal point of the tooth/implant. Distance from mucogingival junction to the marginal gingiva. The mucogingival junction was identified by the roll technique, wherein the mucosa is rolled until the non-movable portion of the attached keratinized tissue is seen.

5.5.3 Calibration of the examiners

To calibrate the examiners, all the study clinical periodontal measurements (PI, BOP, PD and KT) were taken by each examiner in a volunteer undergraduate student. Furthermore, in order to calibrate the dimensions of the graft a piece of a cut tongue depressor was used.

The calibration exercise between the three experienced operators who performed the clinical measurements showed good intra- and inter-reproducibility at the start of the study with an Intraclass Correlation Coefficient (ICC) of 0,81. This calibration exercise was repeated several times during the follow-up period while maintaining reproducibility.

5.6 Intervention to the sample

5.6.1 Initial clinical procedure

After patient inclusion, an oral prophylaxis (oral hygiene instructions, ultrasonic instrumentation and coronal polishing) was performed one week before the surgery. The augmentation procedure was performed 6 weeks after implant placement on implants that were placed according to a transmucosal protocol or at the time of abutment connection (12 weeks after) in those implants that were placed in a submerged fashion.

5.6.2 Surgical procedure

5.6.2.1 Recipient site

Postgraduate students from periodontal department, supervised by experienced faculty professors (AS and JN) performed all surgeries. After the measurements of the clinical periodontal parameters (PI, BOP, PD and KT), intraoral optical scanners were performed after the healing abutments were secured in place. Therefore, in one-stage implants the intraoral scan was performed immediately,

whereas in two stage implants a minimum crestal incision was performed prior to the scan, which allowed the seating of the healing abutment.

The surgical procedure was performed as follows. In brief, intracrevicular incision at the buccal side of the implant extending in one adjacent tooth for each side was placed and a partial-thickness mucosal flap was raised. The incision reached beyond the junction of the attached and lining mucosa. Periosteal releasing incisions were made to assure tensionless closure. At this time point the sealed envelope was opened and group allocation communicated to the surgeon.

After de-epithelization the connective tissue was secured with an absorbable 5-0 suture (Vicryl, Johnson & Johnson, Woluwe, Belgium) in the buccal aspect by means of cross-mattress sutures. Single interrupted sutures were used to approximate the mesial and distal flap margins (Figure 3).

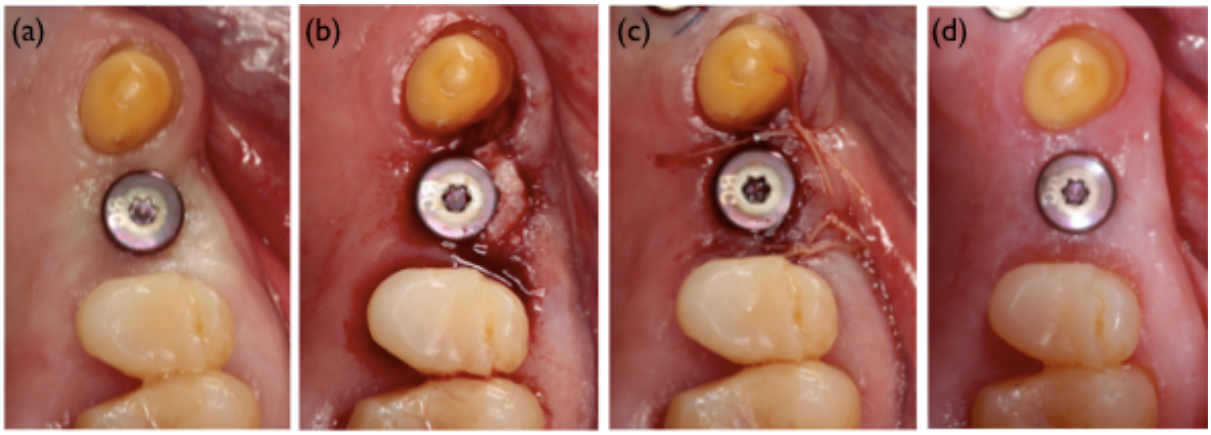

Figure 3. Surgical procedure at recipient site. (a) Clinical situation at baseline. (b) After intrasulcular incisions a partial-thickness flap was raised at the buccal aspect and a SCTG positioned. (c) Graft and flap were secured with sutures, which were removed at 10 days. (d) Clinical result at FU-3.

5.6.2.2 Donor site

A double-bladed scalpel handle 1,5mm (SKU 10-130-05D, Hu-Friedy, USA) was used in both areas in order to obtain the same thickness in each graft.

- Palate (CG)

Two 15 blades were placed at the scalpel handle and the double incision was made approximately 2 to 3 mm apical to the gingival margins of the first and second premolar. The incisions were carried far enough to provide the standardized graft dimensions (12mm in length and 10mm in height). The donor tissue was removed from the palate with care to avoid tearing or damaging the tissue (Figure 4).

- Tuberosity (TG)

Using two 12 blades a double incision was made from the distal of the terminal tooth extending distally in the tuberosity (Figure 4). A second incision was made perpendicular to the linear incision at a distal point, which joined the two linear incisions and extended to the mucogingival junction buccally and into palatal mucosa to the point where the palatal flap was thinned. The initial tracing incision was extended apically to bone. Then the graft was removed.

In both groups the graft was de-epithelized, its measurements were standardized (10mm height, 12mm length and 1,5mm thick) and measured by a blind examiner. When the dimension of the graft allowed it, a tissue sample was obtained and destined for histological analysis. Afterwards cross-mattress sutures were used to approximate the wound margins in the donor area.

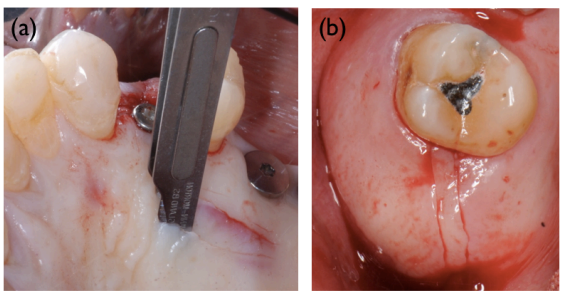

Figure 4. Surgical procedure at donor site. (a) At CG tissue was harvested from the premolar area of the palate. However at TG, the graft was harvested from the tuberosity region (b).

5.6.3 Postoperative care

Amoxicillin 500mg/8h/7days, Ibuprofen 600mg/8h/3days and 0.12% Chlorhexidine with 0,05% CPC solution 2 times daily for 2 weeks were prescribed. In case of allergy to penicillin, Clindamycin 300mg/8h/7 days was prescribed. Application of ice packs was recommended for 4 hours post surgery at interval periods of 30 minutes. Furthermore, all patients were instructed to discontinue tooth brushing during 2 weeks, to avoid trauma or pressure at the surgical site. Suture was removed at 10 days after the surgical procedure. After this period, the patient resumed mechanical tooth cleaning of the treated areas using a soft toothbrush.

During the next 3 months the healing abutment was always connected. A provisional denture or Maryland fixed prosthesis could be worn during this time. Whether a Maryland or removable denture was used, care was taken to avoid pressure in the grafted area.

5.7 Statistical analysis

Continuous variables were shown as mean ± standard deviation for normally distributed data or median (and interquartile range) for non-normally distributed data and categorical variables as proportions. Shapiro test was used to analyse normality distribution. Differences according study groups were analysed using U Mann Whitney test with quantitative variables. Linear relationships were tested through Spearman correlations. Changes post versus pre treatment were calculated by subtracting the values after the 3- months intervention period from the values before the period and differences were analysed using the Mann-Whitney U test.

Inter and intra observer reliability for the different measurements were checked using the Intraclass Correlation Coefficient (ICC) for quantitative variables.Two-sided p values of less than 0.05 were considered to indicate statistical significance. Statistical analysis was performed with the statistical software package SPSS v-22 (Armonk, NY:IBM corp).

RESULTS

6.RESULTS

6.1 Descriptive analysis

A total of 32 patients were entered in the clinical trial having fulfilled all inclusion criteria. Sixteen patients with a mean age of 50,47 ± 13,61 years were allocated to CG (nine females, seven males) and 16 patients with a mean age of 55,44 ± 8,00 to the TG (six females, ten males). Four patients contributed with 2 implants so a total of 36 implants were treated. Two patients were excluded from the study on the basis of refusal to attend follow-up appointments. In one patient the superimposition was not possible. Therefore, 29 patients with 33 implants were evaluated. Patient demographics are shown in table 3-4.

	CG	TG	TOTAL-MEAN
N	16	16	32
Gender (Male/Female)	7/9	10/6	
Implants (*N*/%)	18/50%	18/50%	36
Drop out (Patient/Implant)	3	0	29/33
Age (mean ± standard deviation)	50,47 ± 13,61	54,44 ± 8,0	53,2 ± 11,0

Table 3. Patient demographics.

Implants treated were mainly located at the maxilla (66,66%) and anterior region (72,22%) in both groups. Two stage healing modus represented the 55,55% of the sample, whereas one stage was 44,44% of the sample. Implant location and healing modus are represented at table 5.

	CG	TG
Location		
Maxilla	11	13
Mandible	7	5
Anterior implant (15-25)	12	14
Posterior implant	6	4
Healing Modus		
One stage	9	7
Two stage	9	11

Table 4. Distribution of the implants treated.

Also, 20 histological samples were obtained (11 tuberosity and 9 palate). During the study no postoperative complications were observed in any case except those inherent to oral surgery, such as moderate inflammation and edema during the first few days.

6.2 Primary outcome analysis

The statistical anaylsis with Shapiro Wilks test indicated a non normal distribution of the sample. Therefore non-parametric test (U-Mann Whitney) were applied to compare both groups.

Changes in linear measurements between baseline and FU-3 were evaluated from 1 to 7mm apically to the healing abutment and measured from the preoperative to postoperative soft tissue profile.

Results were evaluated on patient and implant level analysis. For the patient level analysis, one implant was randomly selected in those patients who contributed with 2 implants. No statistical significant differences were found at any point regarding horizontal contour changes between baseline and FU-3. The median horizontal contour increase in CG amounted to 0,59 (0,35-0,81) mm, whereas the

TG obtained 0,75 (0,57-0,97) mm (p 0,13). Results of patient level analysis are shown in table 5, 6 and figure 5.

	CG (mm)		TG (mm)		
	Mean ± SD	Median (Q1-Q3)	Mean ± SD	Median (Q1-Q3)	p-value
1mm	0,58 ± 0,31	0,52 (0,40-0,76)	0,64 ± 0,43	0,51(0,31-0,94)	0,99
2mm	0,83 ± 0,42	0,83 (0,64-1,05)	0,84 ± 0,39	0,87 (0,48-1,12)	0,94
3mm	0,75 ± 0,43	0,83 (0,42-1,05)	0,81 ± 0,39	0,83 (0,40-1,12)	0,86
4mm	0,69 ± 0,,36	0,71 (0,39-1,00)	0,76 ± 0,42	0,79 (0,45-1,12)	0,73
5mm	0,46 ± 0,30	0,44 (0,16-0,74)	0,76 ± 0,45	0,85 (0,36-1,20)	0,16
6mm	0,39 ± 0,24	0,37 (0,24-0,64)	0,77 ± 0,36	0,90 (0,39-1,09)	0,08
7mm	0,22 ± 0,13	0,16 (0,13-0,37)	0,75 ± 0,30	0,81 (0,43- 1,02)	0,10

Table 5. VG Patient level analysis. Results for each mm. Variables in mm Mean ± SD/Median IQR.

	MEAN CHANGES
CG	0,55 ± 0,30 / 0,59 (0,35-0,81)
TG	0,74 ± 0,29 / 0,75 (0,57-0,97)
p value	0,13

Table 6. VG Patient level analysis. Mean values. Variables in mm Mean ± SD/Median IQR.

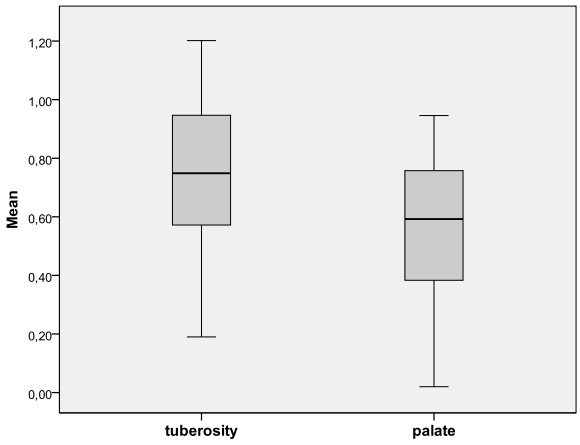

Figure 5. Box plot showing VG data of patient level analysis.

The implant level analysis showed a statistically significant difference between both groups at 6mm apical to the healing abutment favouring the TG, being the median values 0,94 (0,4-1,07) mm, while CG group obtained 0,37 (0,24-0,64) mm (p 0,04) (table 7). The mean changes between baseline and FU-3 soft tissue profile were not statistically significant different between groups, CG revealed a median gain of 0,59 (0,40-0,86) mm whereas TG resulted in 0,75 (0,53-1,01) mm (Table 8 and Figure 6). Although it did not reach significance the VG was more pronounced at TG in both analysis.

	CG (mm)		TG (mm)		
	Mean ± SD	Median (Q1-Q3)	Mean ± SD	Median (Q1-Q3)	p-value
1mm	0,56 ± 0,31	0,52 (0,34-0,76)	0,67 ± 0,42	0,52 (0,31-0,98)	0,67
2mm	0,77 ± 0,44	0,83 (0,41-1,05)	0,80 ± 0,42	0,87 (0,45-1,09)	0,80
3mm	0,75 ± 0,40	0,83 (0,53-1,01)	0,83 ± 0,38	0,83 (0,42-1,14)	0,75
4mm	0,65 ± 0,34	0,70 (0,39-0,93)	0,77 ± 0,44	0,79 (0,35-1,12)	0,48
5mm	0,59 ± 0,49	0,53 (0,21-0,82)	0,80 ± 0,44	0,87 (0,39-1,21)	0,24
6mm	0,39 ± 0,24	0,37 (0,24-0,64)	0,81 ± 0,33	0,94 (0,4-1,07)	0,04
7mm	0,22 ± 0,13	0,16 (0,13-0,37)	0,70 ± 0,27	0,67 (0,46- 0,97)	0,06

Table 7. VG Implant level analysis. Results for each mm. Variables in mm. Mean ± SD/Median IQR.

In both analyses it can be observed that major differences between groups remained at 5, 6 and 7 mm apical to the healing abutment. At these areas, while TG is able to maintain similar values as 1, 2, 3 and 4 mm; CG decreases its values from 5 to 7mm apical to the healing abutment.

	MEAN CHANGES
CG	0,56 ± 0,29 / 0,59 (0,40-0,86)
TG	0,73 ± 0,30 / 0,75 (0,53-1,01)
p value	0,14

Table 8. VG Implant level analysis. Mean results. Variables in mm Mean ± SD/Median IQR.

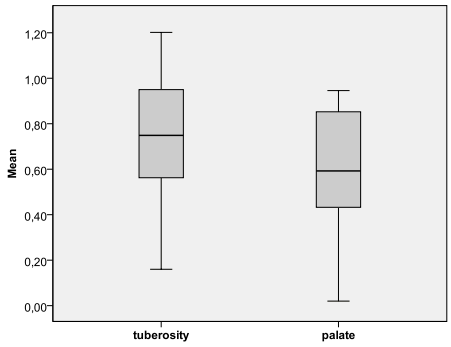

Figure 6. Box plot showing VG data of implant level analysis.

The possible influence of the implant healing modus on the volume changes was evaluated. No statistical significant differences were observed between one or two stage healing modus regarding VG meaning that healing modus has not influence on VG (Table 10).

Healing	Mean ± SD
One stage	0,55 ± 0,32
Two stage	0,57 ± 0,28
p-value	0,95

Table 9. Differences of VG regarding healing phase.

6.3 Secondary outcome analysis

6.3.1 Histology

6.3.1.1 Descriptive Histology

Twenty biopsies (9 control group, 11 test group) were taken from the harvested tissue. The soft tissue biopsies were only taken when the harvested tissue had

greater dimensions that the minimum standardized (12mm in length). For this reason, only 20 patients underwent histologic examination. There were no limitations in terms of size for the tissue biopsies. In some instances, the harvested tissue was only made up of epithelium and lamina propria. There were no evident differences from the tissue analysed in the test and control groups. Both groups presented with a well-defined orthokeratinized epithelial layer and marked rete-pegs that protruded into the connective tissue area. The lamina propria presented in both groups with a well-vascularized tissue and numerous blood vessels that were relatively small and well distributed within the dense collagen fiber network (Figure 7). In those biopsies that extended further apically into the harvested tissue the submucosal portion could be analysed, and bulks of adipocytes and few glandular cells were found to be present. This was the case in 3 specimens from the CG and no specimens in the TG.

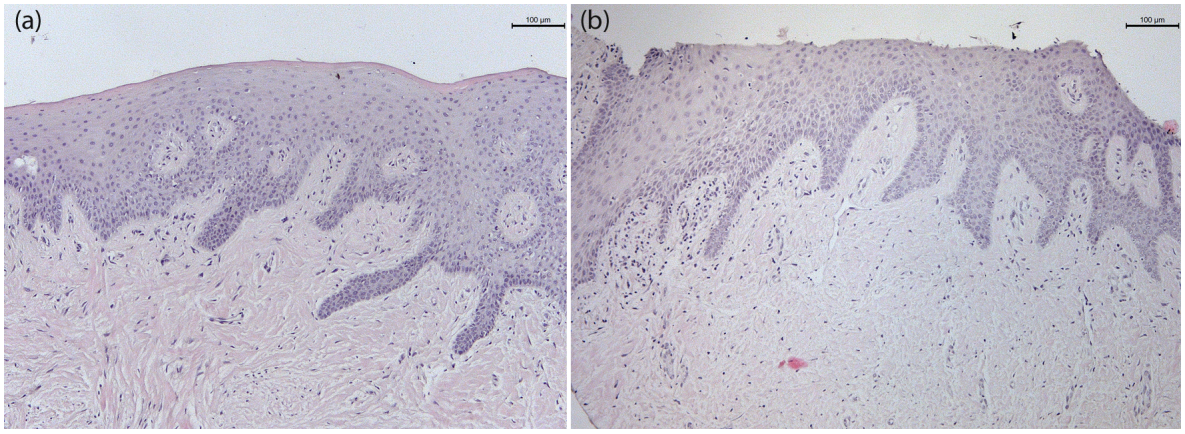

Figure 7. Descriptive histology. (a) Hematoxylin-eosin staining of a tuberosity sample (a) and a palate sample (b). No significant differences were observed in the histological observation.

6.3.1.2 Immunohistochemistry

Different parameters related with collagen turnover were analysed. In this context, LH 2b and MMP 1-2 were compared between CG and TG. Also, parameters

related with epithelium features (CYT) were analysed to evaluate possible differences between CG and TG.

A strong expression of LH 2b was observed at the deepest area of the epithelium, which is described as basal epithelial layer, but also in the lamina propria. In this region LH 2b was observed in the collagen fibers of the superficial layers, especially in the papillary portion, which shows finger-like projections with the overlying basal epithelial layer. Those proteins are expressed in the cellular cytoplasm. No evident differences were observed when analysing samples of CG and TG (Figure 8).

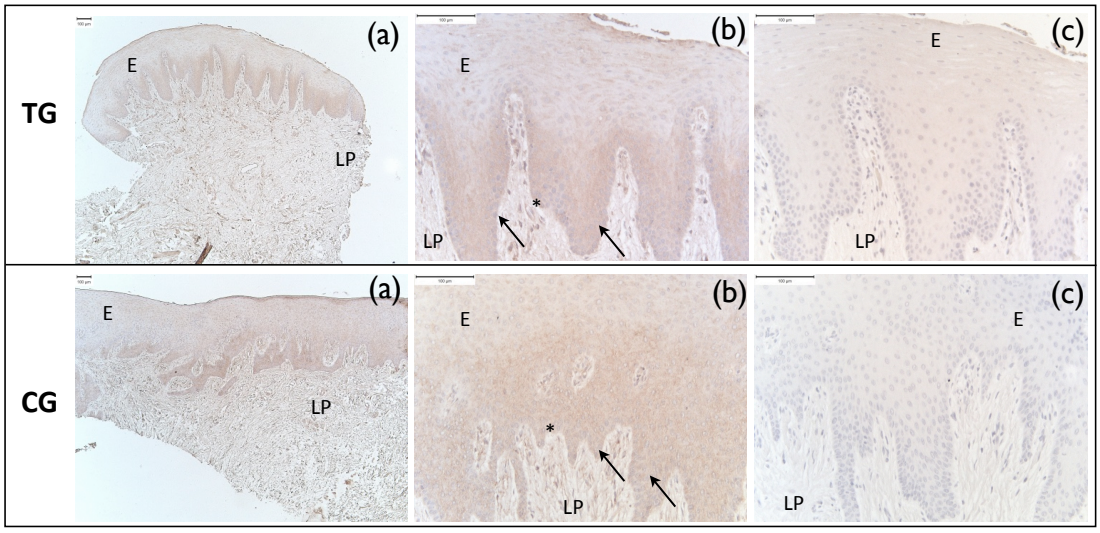

Figure 8. LH 2b analysis. a) Immunohistochemistry of sections (TG and CG) incubated with primary antibody for LH2b at magnification 5x. b) Same sections at magnification 20x. c) Control samples without primary antibody at magnification 20x. E= Epithelium. LP= Lamina propria. *=Basal epithelial layer.

In terms of MMP 1-2, expression was observed in the basal epithelial layer as well as in some collagen fibers of the reticular portion of the lamina propria. The immunohistochemistry analysis revealed a very weak reaction of a few cells for MMP 1 primary antibody in both groups, with no significant differences. In contrast, MMP 2 had some positive reaction, especially in the epithelial cells adjacent to the basal lamina (Figure 9-10).

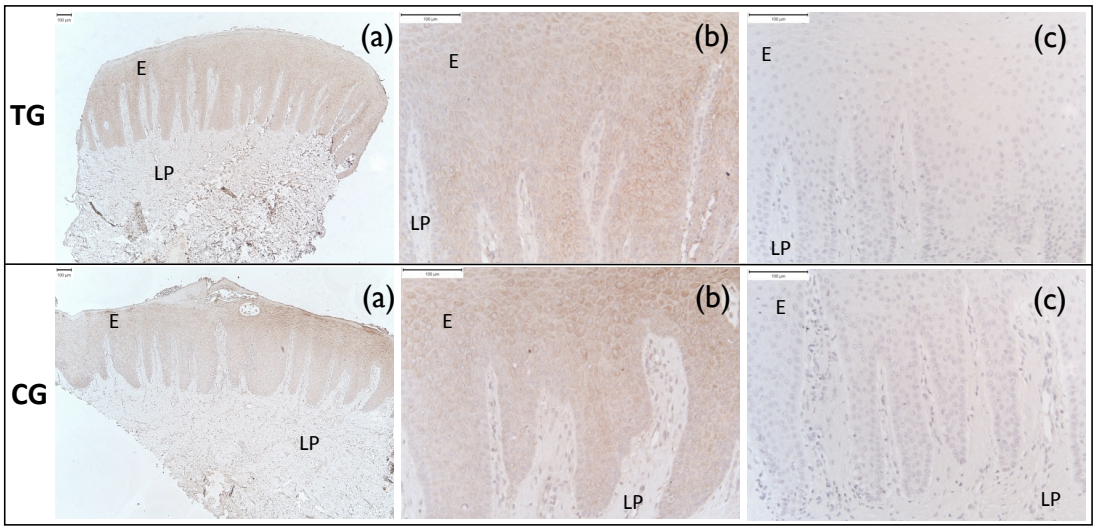

Figure 9. MMP 1 analysis. a) Immunohistochemistry of sections (TG and CG) incubated with primary antibody for MMP 1 at magnification 5x. b) Same sections at magnification 20x. c) Control samples without primary antibody at magnification 20x. E= Epithelium. LP= Lamina propria.

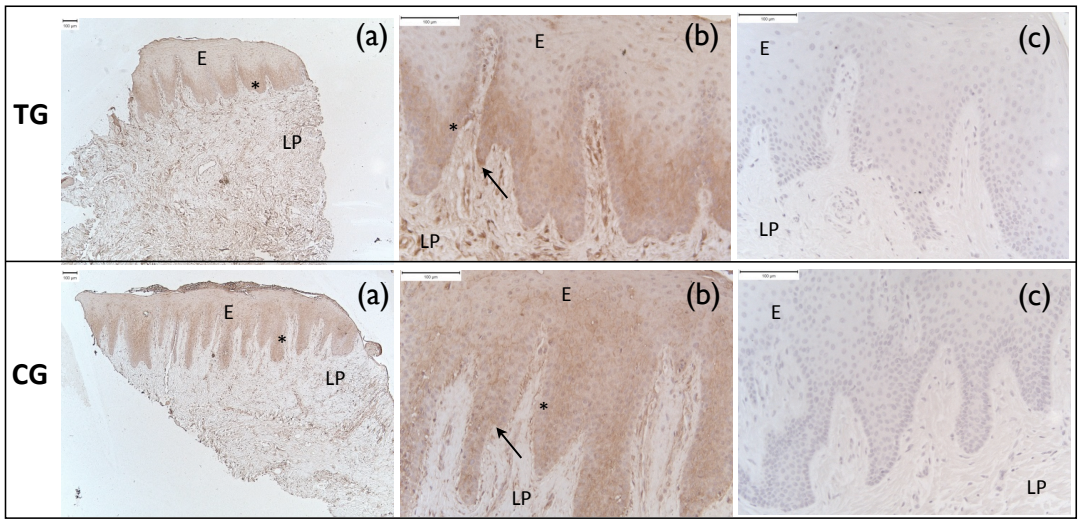

Figure 10. MMP 2 analysis. a) Immunohistochemistry of sections (TG and CG) incubated with primary antibody for MMP 2 at magnification 5x. b) Same sections at magnification 20x. c) Control samples without primary antibody at magnification 20x. E= Epithelium. LP= Lamina propria. *=Basal epithelial layer

Monoclonal antibodies against CYT 4-10-13 were also used to analyse CYT expression. CYT are intermediate filaments proteins located at the epithelium

layers. CYT 4 polypeptide is typically observed in high quantity in non-keratinized epithelium, such as alveolar mucosa; whereas keratinized gingiva does not express this polypeptide. Absence of reaction was observed in palate and tuberosity samples when analysing CYT 4. No differences were observed when comparing with controls, which were not incubated with primary antibody (Figure 11).

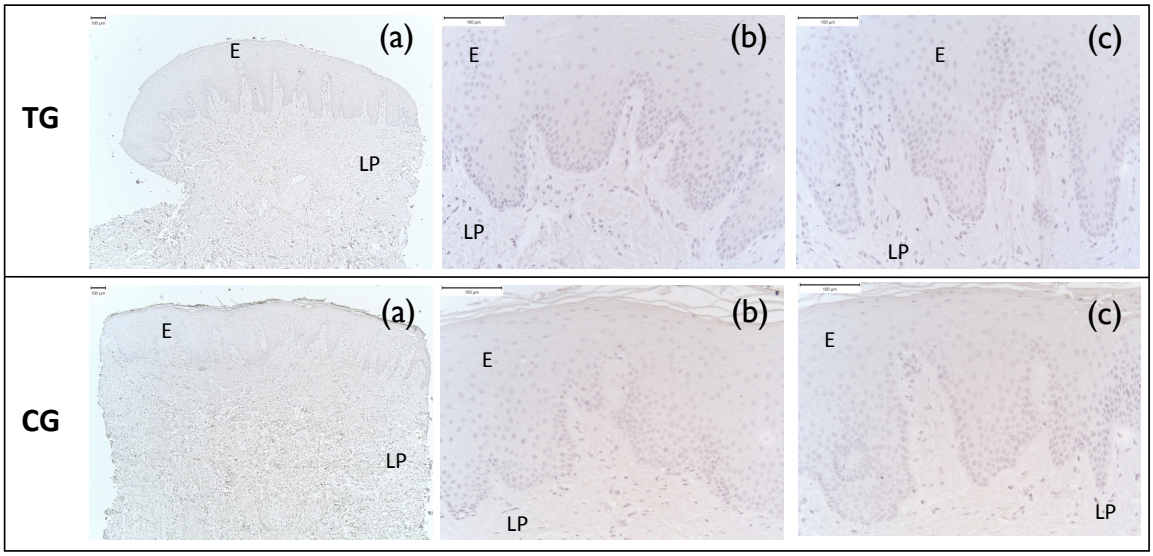

Figure 11. CYT 4 analysis. a) Immunohistochemistry of sections (TG and CG) incubated with primary antibody for CYT 4 at magnification 5x. b) Same sections at magnification 20x. c) Control samples without primary antibody at magnification 20x. E= Epithelium. LP= Lamina propria.

On the other hand, CYT 10 is a characteristic feature of keratinized epithelium. The results showed in biopsies were opposite to those obtained for the CYT 4 analysis. In this case, samples of both experimental groups displayed a high positive CYT expression in all epithelial layers without significant differences (Figure 12). Also a strong positive reaction was obtained when CYT 13 primary antibody was applied. However, both groups showed a similar expression (Figure 13).

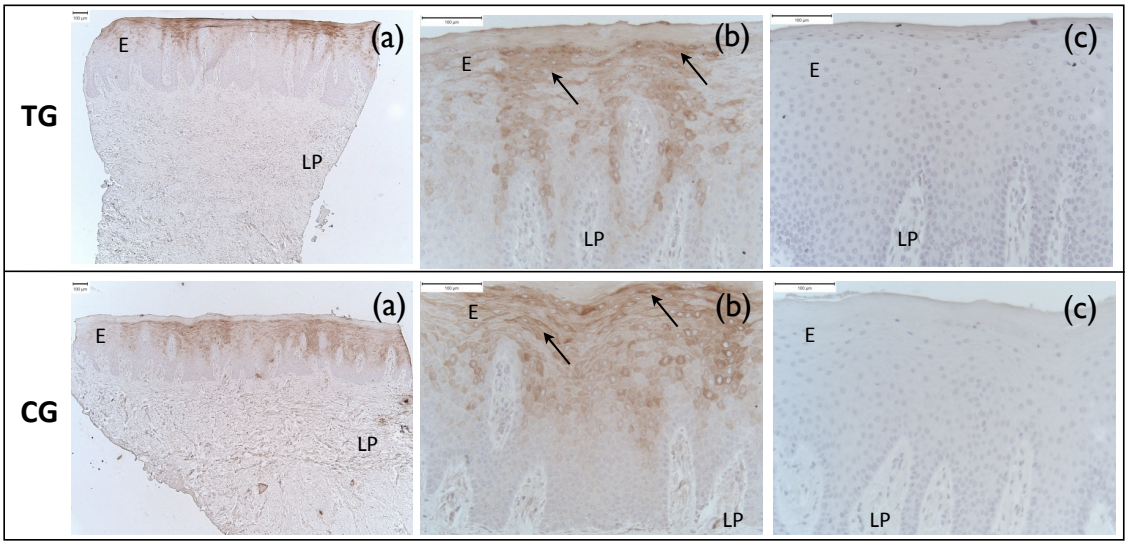

Figure 12. CYT 10. a) Immunohistochemistry of sections (TG and CG) incubated with primary antibody for CYT 10 at magnification 5x. b) Same sections at magnification 20x. c) Control samples without primary antibody at magnification 20x. E= Epithelium. LP= Lamina propria.

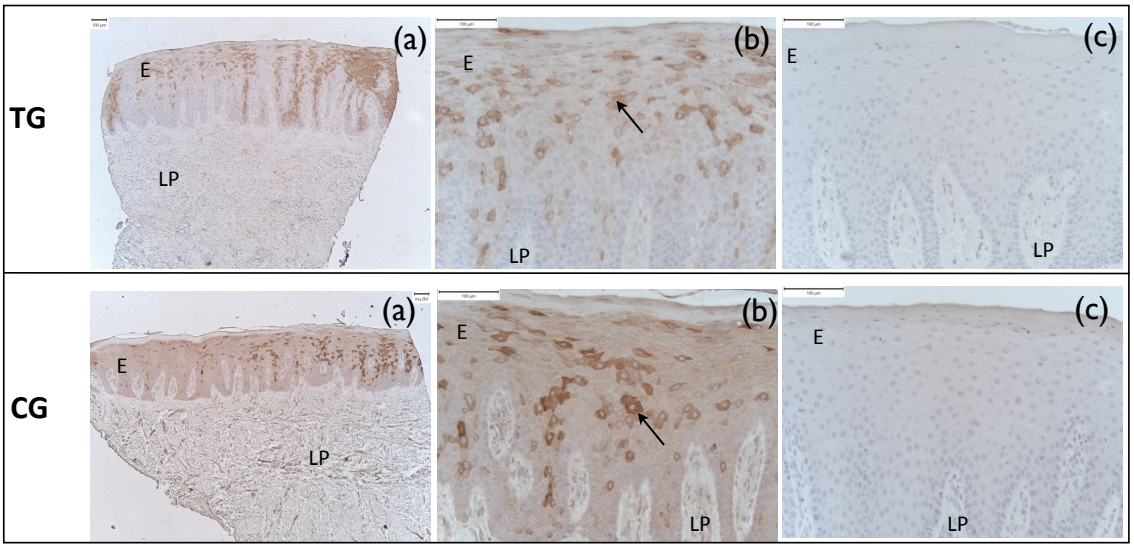

Figure 13. CYT 13 analysis. a) Immunohistochemistry of sections (TG and CG) incubated with primary antibody for CYT 13 at magnification 5x. b) Same sections at magnification 20x. c) Control samples without primary antibody at magnification 20x. E= Epithelium. LP= Lamina propria.

6.3.2 Clinical parameters

No statistical significant differences between groups regarding PI, BI and PD values were observed at baseline and FU-3. Changes in these clinical periodontal parameters between baseline and FU-3 were similar for both groups without statistically significant differences (Table 11).

In terms of PI and PD both groups decrease its values from baseline to FU-3. For BI, CG obtained a negative difference while TG increased its values. All these differences remained no statistically significant.

	BASELINE	FOLLOW-UP	DIFFERENCE
PI			
CG	16,47 ± 3,46	16,6 ± 4,76	0,13 ± 3,33
TG	15,53 ± 2,95	15,83 ± 4,93	0,31 ± 4,15
p value	0,20	0,42	0,99
BI			
CG	10,24 ± 4,28	9,73 ± 4,43	-0,51 ± 3,47
TG	7,83 ± 2,43	8,56 ± 3,71	0,72 ± 2,65
p value	0,12	0,40	0,08
PD			
CG	2,31 ± 0,66	2,28 ± 0,43	-0,02 ± 0,63
TG	2,56 ± 0,52	2,45 ± 0,57	-0,13 ± 0,47
p value	0,35	0,73	0,68

Table 10. PI, BI and PD values. Variables in % (Mean ± SD)

Changes in width of KT were evaluated at implant and adjacent teeth. A statistically significant difference in KT changes at FU-3 was observed favouring the TG, being the median gain 0,83 ± 0,61mm while CG obtained an average gain of 0,22 ± 0,48mm. Table 12 shows KT values.

	BASELINE	FOLLOW-UP	DIFFERENCE
CG	3,99 ± 1,27	4,20 ± 1,60	0,22 ± 0,48
TG	3,67 ± 1,35	4,50 ±1,24	0,83 ± 0,61
p value	0,87	0,26	0,009

Table 11. KT mean values *(Implant and adjacent teeth).* Variables in mm (Mean ± SD).

Further analysis evaluating only the KT changes at implant site was performed. No statistically significant differences were observed at this point, however a tendency for more KT gain in TG was observed (table 13).

	BASELINE	FOLLOW-UP	DIFFERENCE
CG	4,2 ± 1,37	5,07 ±1,48	0,87 ± 0,99
TG	3,72 ± 1,22	5,0 ±1,14	1,28 ± 0,67
p value	0,31	0,79	0,29

Table 12. KT implant site values. Variables in mm (Mean ± SD).

6.3.3 Summary

After analysing the data obtained, it was observed that both groups were able to obtain similar values of VG, without statistical significant differences. Also, no significant differences were found in the histological analysis, confirming that both tissues presented similar histologic features.

Therefore, both null hypothesis (Ha0 – Hb0) have been confirmed, whereas the alternative hypothesis (Ha1 – Hb1) have been refuted.

DISCUSSION

7. DISCUSSION

7.1. Clinical results

This clinical trial was designed to evaluate the differences in soft tissue augmentation when using SCTG of the same thickness and dimensions from palate or tuberosity. No significant differences in terms of buccal soft tissue VG were observed between groups. Until now and from the best of our knowledge, this is the first randomized clinical trial that compares SCTG grafting from palate versus tuberosity around dental implants. Soft tissue augmentation around dental implants is a common procedure used to recover the natural appearance of the dentition, especially in cases where an alveolar process deficiency is observed or when thin tissues are present (9, 60). This investigation demonstrated that in these cases harvesting the SCTG from palate or tuberosity had a similar effect in terms of VG.

There is only one investigation around ridge defects where a comparison between SCTG from tuberosity and SCTG from the palate was made. In this study, Dellavia et al (13) analysed clinical and histological outcomes of patients who received a ridge augmentation by means of SCTG from the palate or from the tuberosity of 3,5mm in thickness. Clinical measurements were taken at baseline, after 1 month, 1 year (concomitantly with plastic surgery in cases who needed due to hyperplasic response) and 9 months after plastic surgery using a periodontal probe with a calibrated surgical stent. Results at 1 year showed a mean increase of thickness of 2,9mm for palate group and 4,7mm for the tuberosity group. Sites which received palate connective tissue graft did not show any hyperplastic reaction and some reduced its volume. Otherwise, sites grafted with connective tissue graft from the tuberosity were dimensionally stable at the first months and then tended to produce an hyperplastic reaction with a non-esthetically outcome. These sites underwent plastic surgery, in order to reduce the non-esthetically appearance. Nine months later, a rebound of 70% of soft tissue was observed. It is difficult to

draw comparison because this study is performed around ridge defects using thicker grafts compared with the present study.

Up to date no studies have been found regarding VG around dental implants involving tuberosity SCTG. For these reason only data of CG will be compared with previous investigations. Studies regarding volume changes around dental implants have been performed. Thoma et al (67) have recently published an investigation where 20 patients in need of soft tissue augmentation were recruited and randomly received a collagen matrix or a SCTG from palate. Soft tissue thickness was evaluated at baseline, 1 months and 3 months using an individualized stent with an endodontic instrument. Three measurements in different locations were performed each time: occlusal, buccal and apical. For the SCTG group changes between baseline and 3 months in terms of soft tissue thickness were 0.8 ± 2,2mm with a median of 0,5 (-1,0; 2.0) on the buccal area and a mean gain of 1,6 ± 2,6mm with a median of 1,8 (-0,5; 3,3) in the apical area. For the collagen matrix group the values were 1,1 ± 1,4 with a median of 1,0 (0,5; 2,0) on the buccal point and 0,9 ± 1,9mm with a median of 0,0 (-0,5; 1,5) for the apical area. No statistically significant differences were found between both treatments in changes at 3 month. The authors concluded that both procedures were effective in increase soft tissue volume. Similar results were obtained in a study (106) where the same collagen matrix was used. The analysis was made by means of 3D superimposition obtaining at 3 months 0,94 (0,66; 1,13) for SCTG and 0,59 (0,25; 1,06) for the collagen matrix.

Wiesner et al (46) performed a randomized controlled clinical trial with a split-mouth design. After bilateral implant placement, one side was augmented with SCTG from the palate while the other was non-grafted. The graft was placed on top of the implant augmenting both buccal and lingual side. After 3 months healing abutments were placed and crowns fabricated. Soft tissue thickness was evaluated using an endodontic file prior to implant placement and 1 year after loading at both buccal and lingual sites. At 1 year the average increase in buccal and lingual sides of grafted areas was 1,20 (0,63) mm, whereas the non-grafted

site experimented a decrease of 0,15 (0,34) mm. Therefore, at 1 year after loading augmented sites were 1,3 (0,50) mm thicker compared with non-augmented areas.

Likewise, retrospective studies have been done evaluating soft tissue changes around dental implants. In this context, Speroni et al (63) evaluated soft tissue changes using individualized acrylic stents, in cases where a free connective tissue graft or SCTG from different areas (tuberosity and palate) were performed. The mucosal thickness was evaluated at different time points. The highest values in thickness were observed at 2 weeks (4,11mm). From this time point (2 weeks) to the last follow up of the study (36 months) a slow decrease in mucosal thickness was observed. Therefore, the mucosal thickness at 4 months was reduced until 2,29mm. After 12 months the values were reduced until 1,75mm compared with the baseline values. Finally at 36 months, the mean mucosal thickness was 1,40mm.

A similar trend was observed in two recent studies that evaluate the soft tissue changes using an ultrasonic device. In the first prospective study (9), thirty-seven patients with single implants in need of horizontal contour augmentation for aesthetic reasons were selected. Following the implant integration a provisional crown was placed and the augmentation procedure performed by means of envelope technique and SCTG from the palate. Immediately after grafting the mucosal thickness increased 1,07mm. At suture removal an additional increase of 0,38mm was observed as a consequence of postoperative swelling. At 3 months the average increase in horizontal contour was 1,09mm, which slightly decrease until 0,97mm at 12 months after the implant placement. The second prospective study used the same protocol as above, (60) in that case the soft tissue profile augmentation amounted to 0,92 ± 0,33mm immediately after SCTG and 0,98mm after 3 months. Again from 3 to 12 months a slight decrease of 0,15 ± 0,19mm could be observed, obtaining a final horizontal increase of 0,83mm at 12 months of implant placement (9 months of SCTG).

In the present study the median increase in VG was respectively 0,59 (0,35-0,81) mm for the CG and 0,75 (0,57-0,97) mm for the TG (p=0,13), which are lower values when compared with previous studies. It is difficult to draw comparisons, but a number of explanations could explain these differences. First of all, in these previous studies the graft was thicker (13) than the present study or its dimensions were not standardized (9, 46, 60, 63, 67, 106). In the present study an effort has made in order to obtain the same dimensions and especially the same thickness in each graft. In a previous study (10) it was reported a significant linear relationship between thickness increase and baseline graft thickness. This may partly explain the results of the present study.

Secondly, the method used to evaluate volumetric changes was not the same in all the studies. Some of them used an endodontic file with (13, 63, 67) or without (46) a customized stent, while others (9, 60) used an ultrasonic device or a 3D superimpositon after scanning dental casts (106). In the present study an intraoral optical scan has been used together with a superimposition software. It seems, as it will be explained further on in this discussion, that digital techniques could be more reliable than clinical measurements (107).

Thirdly, the surgical procedure used in some studies (9, 60) where a provisional crown is immediately placed after the surgery differs from the present study where no provisional has been placed. This may interfere with the increase of thickness leading to a more soft tissue augmentation. It has been reported in previous investigations that after abutment and crown placement a mean increase from 0,69 to 0,9mm in terms of buccal mucosal thickness can be expected (108, 109).

Finally, in some of the previous papers the surgical interventions are performed by experienced surgeons (46, 60, 67, 106) while in the present study, although the surgical approach was supervised by experienced faculty professors, it was performed by postgraduate students. It is known from the literature, that lack of expertise and skills in treatments as technique-sensitive as periodontal plastic surgery may compromise negatively the results (110).

According to the results of the present study, no statistical significant differences in terms of thickness gain were observed between both groups. This is in accordance with a systematic review (70) and a consensus report (110) where after the review of the available literature, it was concluded that there is no evidence of which technique is more effective in soft tissue augmentation around implants. Nevertheless, more favourable outcomes were observed in patients who received SCTG harvested from the tuberosity. This may be explained by studies such as Zhur et al (11) who reported that the SCTG from the tuberosity area is a very dense and coarse tissue which seems to contain more collagen and less fat and glandular tissue, compared to the anterior lateral palate. It must also be taken into consideration that the strict standardization of the graft thickness at 1.5mm may have homogenized graft characteristic, particularly, in the most coronal aspect were lamina propria was also harvested in the CG.

Interestingly, the major differences between both groups were observed from 5 to 7mm apical to the healing abutment favouring TG. This outcome could be expected, since it is known from histological palatal studies (86, 87) that the closest area to the palatal gingival margin contains the higher amount of lamina propria compared with apical areas, which seems to contain more glandular and fat tissue. In contrast, the tuberosity appears to contain much more lamina propria in its whole dimension (11). It can be assumed that areas with more lamina propria would be less prone to shrinkage leading to a more VG (86). This would explain the differences found at the apical area, where TG performed better when compared to the CG.

In terms of KT gain, a statistically significant difference was observed between groups favouring the TG with KT gain of 0,83±0,61mm while CG obtained 0,22±0,48mm. Ouhayoun et al (88) performed an study where a thick palatal FGG, was split into two thinner grafts, a superficial epithelium-connective tissue graft and a deep connective tissue graft. After the transplantation in a mucosal bed, sites receiving the superficial epithelium-connective tissue graft showed

histological properties of keratinized mucosa. Otherwise, sites grafted with deep connective tissue mostly showed characteristics of non-keratinized gingiva. In contrast, previous studies (111-113) demonstrated that the connective tissue graft is able to induce keratinization when grafted. Although, it is important to underline that connective tissue grafts from these previous studies (111-113) were harvested from the superficial layers of lamina propria, which contains a large amount of connective tissue. In the other hand, the deep connective tissue grafts of Ouhayoun's study were harvested from deeper areas, which may content more fat and glandular tissue. From the results of these investigations it may be assumed that different palatal layers (lamina propria and submucosa) have different molecular signals which may interfere in the formation of the newly tissue. Also, it appears that areas with more lamina propria could gain more KT. This may explain the results observed in the present investigation where tissues that may content more lamina propria (TG)(82), obtained better values when KT was concerned.

When analysing changes around the implant (CG 0,87 ± 0,99mm / TG 1,28 ±0,67mm), results of the present study are higher than those reported in other investigations (10) where a statistical significant mean KT increase of 0.57 ± 0.41 mm was obtained after performing a coronally advanced flap in combination with SCTG from the palate in a different clinical scenario (treatment of buccal soft tissue deficiencies). Other studies obtained higher values such as the study of Basegmez et al (114) where after performing a soft tissue augmentation by means of FGG or vestibuloplasty a KT increase between 1,2-2,4mm was obtained. In agreement with these study, Bruschi et al (115) obtained an average increase of 5,1mm using similar surgical techniques. As far as the gain of KT around teeth is concerned Oates (116) made a systematic review including thirty-two randomized controlled trials and concluded that a mean gain of 1,5mm can be expected when using SCTG. Our results are lower compared with the previous studies reported. One explanation might be that these studies are performed in a different scenario, around teeth or using different surgical techniques while our study is performed around implants and with SCTG. Although it is still a controversial issue, long term

studies seem to indicate that KT around dental implants have an important effect to prevent biological complications (32). Therefore, when the lack of volume is associated with a limited KT, SCTG from the tuberosity may be a better option.

7.2. Histological results

According to the results of the present study no major differences between CG and TG samples were observed in terms of descriptive histology. Both tissues presented a well-defined orthokeratinized epithelium layer with rete-pegs, which interlocked with the underlying lamina propria. The connective tissue was dense and well vascularized in both groups, with similar amount of blood vessels and collagen fibers. A previous study (13) evaluated palate and tuberosity from an histological point of view. The results of this study, reported that all samples showed a healthy structure, without inflammation and normal epithelial structure. Although, collagen content was similar for both groups (tuberosity 80.91% ± 8.77% vs palate 75.57% ± 7.68%), morphologic differences were observed. The connective tissue in the palatal lamina propria seemed to be looser and highly vascularized; while in the tuberosity biopsies it appeared to be denser and poorly vascularized. Also, in other studies the tuberosity was described as a dense tissue with less fat and glandular tissue in its deeper layers (11, 12, 82).

Histological tuberosity studies (82) revealed a mean dense fibrous lamina propria of 1.6 to 2 mm on the buccal area, 2.5 to 4 mm on the ridge and 2 to 3.5 mm on the lingual aspect. Interestingly, the facial area of the tuberosity showed only features of dense fibrous connective tissue while the lingual side was characterized by a dense fibrous connective tissue with a submucosa underneath. A similar amount of lamina propria was obtained in a study of the palatal area (85). After analysing 30 palatal connective tissue grafts harvested with a deep pair of parallel incisions, the mean depth of the lamina propria was 3,2 mm (65,2% of the graft), and the mean depth of submucosa 2,0 mm (34,8% of the graft). However, the size of the lamina propria ranged from 1,1 to 6,3mm, and the % of lamina propria in the total graft from 21,1 to 100%. A high interindividual variability was

observed, while in some cases the grafts was entire lamina propria without submucosa, others presented only a minimal portion of lamina propria. Similarly, Bertl and colleagues (86) obtained a high variability when analysing palate samples. The proportion of fat/glandular tissue (FGT) ranged from 0.04% to 73.8%, and fibrous connective tissue from 23.2% to 93.3%. Likewise, the portion of lamina propria containing ≤ 25% of FGT had a thickness from 0.2 to 2.8 mm and lamina propria containing ≤ 50% of FGT presented a thickness from 0.3 to 3.3 mm, meaning a large interindividual variation. Even though there is limited scientific evidence, it seems reasonable to assume that tuberosity tissue could be less variable in terms of lamina propria content. Furthermore, it seems that the submucosa content is smaller in the tuberosity area, particularly, at the buccal area. These may be the most obvious difference between both tissues. In the present study not all the samples presented the entire connective tissue layer, so no measurements of lamina propria and submucosa could be done. As has been explained, the biopsies were taken only when an excessive graft dimensions was harvested.

The differences with Dellavia's study (13) may be related with the graft thickness and palatal tissue variability. In the previous study (13), a graft of 3,5mm thickness was harvested, while in the present investigation the thickness was limited to 1,5mm. As has been explained above (86) deeper palatal layers contains a higher amount of fat and glandular tissue. Then it may be hypothesized, that in the present study as the graft was limited to 1,5mm thickness and biopsies sample size were relatively small (average size 1x1x5 mm) only the superficial layer of the palate (which contains the higher amount of denser connective tissue) was harvested. Then, differences with tuberosity were not as significant as in the previous study mentioned, where samples were taken deeper and thicker. Also, the high interindividual variability in the palate histological samples reported in some papers (85) could be another explanation of the differences observed.

In the present study parameters related with epithelium (CYT 4,10,13) and with the underlying connective tissue (LH 2b, MMP-1, MMP-2) were assessed to evaluate

possible differences between palate and tuberosity. The connective tissue parameters evaluated are related with collagen turnover (MMP and LH 2b), as may be hypothesized that tissues with more collagen could obtain higher values in VG (11). However, according to our findings no significant differences were observed between both groups (LH 2b, MMP-1, MMP-2). In agreement there is one study (13), where not statistically significant differences were found between palate and tuberosity samples. In terms of collagen and LH 2b, levels expressed were similar in both groups. Interestingly, MMP-1 mRNA levels decreased in 50% in tuberosity samples, but when analysing the main protein levels this differences were not present. However, the main difference observed was in the LH 2b/Collagen-I mRNA levels, which were four-fold increased in tuberosity samples, even though these differences did not reach statistical significance. These tendencies were not observed in the present study. There are some reasons to explain these disparities. First of all, different analysis techniques were used in both studies. While in the present study, immunohistochemistry analysis was performed, in Dellavia's study the results were based on cell cultures, real time RT PCR, slot blot and SDS Zymography analysis. Finally, as was explained above, different graft dimensions and thickness were used.

LH 2b is an important enzyme related with collagen maturation and extracellular matrix stability. It may be assumed that can influence the collagen content (13, 102). It is a key step in the biosynthesis of collagen cross-links, which are essential for the stability of the connective tissue (117). In the cross-link synthesis, LH 2b produces an overhydroxylation of the collagen telopeptides favouring the formation of pyridinolines cross-links (102). It appears that collagen fibers with these kinds of cross-links may be more difficult to degrade by MMPs leading to its accumulation. This was observed in fibrotic processes (103, 104), but also in gingival overgrowth cases. In these situations, where an excessive collagen accumulation is observed, higher values of collagen type I, MMP-1 and LH 2b were found when compared with healthy patients (101). Collagenases such as MMP-1 and MMP-2 are related with collagen degradation procedures in the periodontal environment (98). This procedure is started with the interstitial collagenase or MMP-1, which cleaves the triple helical structure of the collagen

fibers. Then, a less specific gelatinase, for example MMP-2, recognize the collagen and degrades it (105). It was observed in severe periodontal patients a linear inverse relationship between collagen and MMP, meaning that tissues with less MMP may present more collagen (99). Even though there is limited scientific evidence, it could be hypothesized that if tuberosity tissue may present a higher amount of LH 2b and lower values of MMP, as was observed in a previous study (13), this could lead to a more collagen accumulation and possibly to a more VG. However, according to the results of the present study no significant differences were observed.

CYTs are intermediate filaments proteins, which are involved in functional epithelial aspects (118). As intermediate filaments, CYTs formed part of the epithelial cytoskeleton helping to the cell-cell adhesion. CYTs also take part in the maintenance of integrity and morphology of the epithelium (119). The expression of different CYTs seems to be specific to each type of epithelium. Since it was described that lining mucosa and KT expresses different kinds of CYTs, these parameters have been used in the literature to evaluate tissue characteristics and properties (118). In a classical study it was reported that lining mucosa expresses mainly CYT 4 and 13; while gingiva react positively to CYT 1, 2, 5, 6, 10, 11, 13, 14, 16, 17 (120).

In this context, Garzon and co-workers (118) used the CYT expression to evaluate the tissue obtained after an artificial graft was placed. Comparisons with control human biopsies obtained from the retromolar area were performed. At this area a positive expression of CYT 4, 10 and 13 was found. In contrast, studies (121) evaluating the oral gingival epithelium at the buccal area of premolars, where KT was present, reported a highly positive reaction to CYT 10, less positive reaction to CYT 13 and negative expression for CYT 4. These results are in agreement with the present study where a strong expression of CYT 10-13 and a negative reaction to CYT 4 was observed in both groups. Literature (122) showed that CYT expression is really sensitive depending on the area evaluated. In the same palatal gingival epithelium different CYT can be observed depending on the specific

anatomical region. While in the oral site of the keratinized palatal epithelium CYT 1-10 are mainly expressed, in the oral sulcular epithelium attaching the junctional epithelium of the same area, CYT 4-13 may be also observed. Discordances with previous study mentioned (118) may be due to the features of the different areas evaluated.

In a classical study (88), a superficial epithelium-connective tissue graft was compared with a deep connective tissue graft. In the superficial graft cases a higher amount of CYT 10 was observed, while in cases of deep grafts a positively reaction to CYT 4 was detected. In terms of CYT 13, all superficial graft biopsies showed some positive reaction at suprabasal layers, while the deep graft samples react positively only in some cases. These differences were not observed in the present study, where biopsies were only taken when the harvested tissue had greater dimensions that the minimum needed after standardization. Then, only 3 samples showed deep layers (submucosa) of gingiva at the present study. Therefore, a proper comparison was not possible. Also, the technique used in the previous study was gel electrophoresis, whereas in the present study immunohistochemistry was performed.

Clinical results of the present study showed a tendency of more KT gain and a higher VG in the TG. According to the histological outcomes, no noticeable differences were found either for the descriptive histology or the immunohistochemistry analysis. Then, it could be hypothesized that probably the epithelium and connective tissue of the palate and the tuberosity areas have similar features, and the mean difference between both tissues remains in the amount of lamina propria and submucosa as was previously described (82, 86).

7.3 Techniques for volume gain assessment

Different methods have been used to assess volumetric changes. Ten years ago Studer (55) used a projection Moiré system; nearly a decade later, Fickl (123) utilized a 3D camera to scan casts. Gonzalez and co-workers (124) used optical

cast scan to assess volumetric and profilometric changes in cases where a SCTG at pontic sites was performed to improve an alveolar process deficiency. The accuracy of the scanner employed at this study was ±6 µm, and its repeatability ±10 µm.

The accuracy of the scanner used (Lava Chairside Oral Scanner C.O.S., 3M ESPE, Seefeld, Germany) has been evaluated in previous studies comparing traditional digitalized impression and digital impression. In the study of Syrek et al (125), crowns were fabricated with both systems. Results showed a significant difference between groups with better values for the digital impression group. Therefore, a median marginal gap in the conventional impression group was 71 microns (45 microns; 98 microns), whereas 49 microns (32 microns; 65 microns) for the digital impression group. A similar study (126) was performed, where a conventional system to prepare a crown was compared with a fully digital method. The results showed a mean marginal gap of 52.66 microns with the conventional system compared with 14.98 microns using the full digital system, when the crown preparation was round shoulder. In cases where the crown preparation was chamfer, mean values of 64.06 microns were registered for the conventional method, while 18.45 microns were observed for the digital method. It was concluded that intraoral scanner displayed significantly smaller marginal gaps.

In contrast, some studies showed no difference in marginal accuracy between both methods. Almeida et al (127) reported no significant differences when evaluating the marginal fit of a four-unit zirconia fixed dental prostheses using the digital or conventional technique. The mean marginal fit was 63.96 and 65.33 µm, respectively. Even though differences in internal fit showed significance, being 58.46 and 65.94 µm, respectively. In agreement with these results, Seelbach and co-workers (128) observed a non statistical difference in marginal and internal fit when evaluating crowns obtained by digital and conventional impressions.

Another study (129) evaluated the accuracy of different scanners in an acrylic in vitro model. Digital impressions were taken using iTero (ITE), cara TRIOS (TRI),

CEREC AC with Bluecam (CBC), and Lava COS (COS) systems. Two measurements were evaluated, internal and marginal fit. Results showed mean marginal discrepancies of 90 microns for ITE, 128 microns for TRI, 146 microns for CBC, and 109 microns for COS. While the mean internal discrepancies were 92 microns for ITE, 106 microns for TRI, 84 microns for CBC, and 93 microns for COS. Differences among impression systems were statistically significant, favouring the ITE and TRI methods. Even though they concluded that all fabricated restorations showed acceptable marginal and internal gap sizes.

It seems, that these digital methods could be at some point better than the traditional ones. A more recent investigation (107) has evaluate the reliability of 4 methods when measuring gingival recessions and papilla heights. In the method A the measurements were done by means of a direct clinical measurements with periodontal probe; the method B consisted in a direct measurements on cast model using a caliper; the method C consisted in digital measurements of intraoral scans and the method D a digital measurements of digitized cast models. Results showed that the highest agreement between all methods was found in the digital ones (C and D), concluding that the use of both digital technologies (direct intraoral impression and digitized cast model) improved the reproducibility and lowered the intra and interexaminer variability. No differences were found between C and D methods, so the question of which is the most accurate method remained unanswered. In the present study an intraoral optical scan has been used avoiding the dimensional changes of the curing processes.

The software used to perform the superimposition was also used in previous publications (124, 130, 131). The US National Institute of Standards and Technology (NIST) tests showed that this software was accurate to less than 10^{-4} µm in position and radius, and 10^{-4} arcseconds (1/36,000 of a degree) in angle of tilt compared with the official reference values. Similarly, the German physical and technical standardization organization (PTB) affirmed an accuracy of less than 0.1 µm in length and 0.1 arcseconds in angle (124).

7.4 Limitations and future expectations

There are some limitations in the methodology of the present study that have to be taken into account when evaluating these results. These limitations will be discussed:

- Follow-up.
- Negative control group.
- Different implants and healing abutments.
- Number of operators and expertise.
- Clinical parameters not evaluated.
- Future expectations.

7.4.1 Follow-up

There is limited scientific evidence in the field of soft tissue volume augmentation around dental implants using intraoral optical scans or ultrasonic devices. In the literature, there are studies with different follow-up. There is emerging evidence related with the use of a new collagen matrix to achieve VG, which evaluate its results at 3 months (67, 106) but also studies using ultrasonic devices with longer follow-up (12 months) (9). Even though, from a clinical point of view, it seems clear that 3 months is a short-term data and studies with longer follow-up are needed to confirm or not the results of these investigations.

7.4.2 Negative control group

In the present study a negative control group was not evaluated. These would give more importance to the outcomes obtained in cases where a soft tissue augmentation was performed. Even though other similar investigations (67, 106)

did not have a negative control group, there is a recent investigation where volumetric changes of the tissues surrounding implants were evaluated 1 year after loading and these could be used as a reference of negative control group. In this paper (132) two different implant systems were evaluated, one and two-piece implants. Volumetric analysis revealed a mucosal thickness loss of -0.15 mm (±0.20) at 1 mm, -0.06 mm (±0.20) at 3 mm, and -0.2 mm (±0.51) at 5 mm for the two-piece implants group. For the one-piece implants changes were -0.03 mm (±0.35), 0.01 mm (±0.28), and -0.01 mm (±0.51) at the three levels respectively. No statistical significant differences were found between baseline and 1 year for both groups, concluding that minimal changes occurred during the first year. Then, it may be speculated that a negative control group would have presented minimal changes.

7.4.3 Different implants and healing abutments

In the present study different implants and healing abutments were used. It is known that after abutment and crown placement, due to the pressure applied to the gingiva a mean increase of mucosal thickness ranging from 0,69 to 0,9mm may be observed (108, 109). In the present study, no standardization of the healing abutment size was performed; therefore the different width of the healing abutments used may have interfered in part with VG results.

7.4.4 Number of operators and expertise

In the present study although experienced professors supervised the augmentation procedures, these were performed by postgraduate students. Thus as previously discussed could influence the clinical outcomes.

7.4.5 Clinical parameters not evaluated

It is known from mucogingival studies that flap thickness is a significant predictor for root coverage. A linear relationship was described, suggesting that thicker flaps

were more prone to achieve complete root coverage (133, 134). A possible explanation for these outcomes was related with a higher tissue vascularization in thicker gingival flaps. However, studies evaluating VG and gingival biotype around dental implants (9) suggested that gingival biotype did not influence the VG outcomes. Nevertheless, there is weak evidence on these issues and it would have been interesting to evaluate gingival thickness and biotype in the present study.

Also, the methodology used did not allow evaluating the dimensions of the baseline defect. This may affect the results of the present investigation. However, in previous volumetric analysis (106, 124) studies this was not recorded either.

7.4.6 Future expectations

For future research, it would be important to evaluate soft tissue volume stability in a longer follow-up to observe medium and long-term outcomes. Also, it would be interesting to evaluate patient centered outcomes either in terms of aesthetic (PES and WES) and patient discomfort. In the present study this was not evaluated, but clinical experience may indicate that SCTG from tuberosity could cause less patient discomfort. Therefore, it can be hypothesized that achieving the same clinical outcomes SCTG from tuberosity would be a better procedure.

CONCLUSIONS

8. CONCLUSIONS

Within the limitations of this study we may conclude that both procedures were effective in increasing soft tissue volume. Using SCTG of the same dimensions from palate or tuberosity has similar clinical outcomes in terms of buccal thickness gain, with a non-statistically significant superiority in VG and KT towards the TG, at 3 months. Also, there were no evident differences from the biopsies examined in the test and control groups. A longer follow-up is needed to confirm or refute these clinical results.

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ANNEXES

Annex 1: Phd project approval

Universitat Internacional de Catalunya | Escola de Doctorat

Dr. José Nart Molina
Universitat Internacional de Catalunya
Facultat d'Odontologia
C/ Josep Trueta s/n
08195 Sant Cugat del Vallès

Benvolgut Dr. Nart,

Amb la present, li comunico que la Comissió Acadèmica del Doctorat en Ciències de la Salut, en la seva sessió del passat 8 de juliol, i una vegada estudiada la seva sol•licitud va acordar aprovar el projecte de tesi amb títol "Soft tissue volume gain and stability around dental implants after abutment connection surgery using autogenous Sub-epithelial connective tissue grafts harvested from the palate or tuberosity. A randomized propspective clinical study" del doctorand Ernest Rojo Xicart i que sigui admès al període d'investigació del Doctorat en Odontologia.

S'acorda nomenar al Dr. José Nart Molina com a Director i al Dr. Antoni Santos Alemany com a Codirector.

Per altre banda li fem saber que la normativa de la UIC estableix que s'ha d'obtenir una avaluació favorable del Comitè d'Ètica en la Recerca, abans del inici de la investigació. Haurà d'aportar aquest informe en quan l'obtingui.

Finalment la volem informar que per poder tramitar la matrícula es poden posar en contacte amb la Sra. Sònia Soriano (ssoriano@uic.es).

Per qualsevol qüestió que vulguin comentar no dubtin en posar-se en contacte amb nosaltres.

Atentament,

Empar Lorda
Secretaria Comissió Acadèmica
Doctorat en Odontologia
Escola de Doctorat
Universitat Internacional de Catalunya

Barcelona, 17 de juliol de 2014

Annex 2: Ethics approval CEIC

CEIC Comitè Ètic d'Investigació Clínica

CUO Clínica Universitària d'Odontologia | Universitat Internacional de Catalunya

CARTA APROVACIÓ ESMENA N.3 PEL CEIC

Número de l'estudi: PER-ECL-2011-01-NF (Esmena n.3)
Versió del protocol: 1.2
Data de la versió: 21/05/2012
Títol: "Ganancia de volumen y estabilidad alrededor de implantes dentales después de la conexión del pilar utilizando injerto conectivo subepitelial de paladar o tuberosidad. Estudio clínico prospectivo".

Sant Cugat del Vallès, 05 de novembre de 2014

Dr. José Nart

Referència: "Ganancia de volumen y estabilidad alrededor de implantes dentales después de la conexión del pilar utilizando injerto conectivo subepitelial de paladar o tuberosidad. Estudio clínico prospectivo"

Benvolgut Doctor,

Els membres del CEIC de la Clínica Universitària d'Odontologia, els hi agraeixen l'aportació científica en el camp de la investigació i la presentació de l'esmena en aquest Comité per a la seva avaluació.

Valorades les noves aportacions realitzades a l'esmena n.3, sol.licitades pel nostre CEIC, el passat dia 5 de novembre de 2014, li comuniquem que el dictamen final ha sigut FAVORABLE.

Li recordem que, segons la Normativa del Real Decret 223/2004 art. 27, s'haurà de presentar al Comitè d'Ètica d'investigacions clíniques de la CUO, i a través de la Comissió Científica, un informe preliminar mensual del seguiment de l'esmena i un informe final un cop finalitzada aquesta.

Quedem a la seva disposició per a qualsevol dubte o aclaració al respecte.

Atentament,

Dr Magí Brufau
President CEIC

CEIC Comitè Ètic d'Investigació Clínica
5 NOV. 2014
Núm. de registre
S-0008

Annex 3: Information for the patient

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6. DOCUMENT D'INFORMACIÓ AL SUBJECTE PARTICIPANT DE LA TESIS DOCTORAL

Codi del protocol d'investigació: PER-ECL-2011-10-NF
Versió del protocol: 1.2
Data de la versió del protocol: 7 mayo 2012
Data de la presentació del protocol: 20 octubre 2014
Títol de l'estudi: Ganancia de volumen y estabilidad alrededor de implantes dentales después de la conexión del pilar utilizando injerto conectivo subepitelial de paladar o tuberosidad. Estudio clínico prospectivo aleatorizado.

Director/a de la Tesis: Dr. José Nart Molina.
Doctorant: Ernest Rojo Xicart
Tutor/a / Monitor/a: -.
Departament: Periodoncia.
Línia d'investigació: Aumento y estabilidad de volumen en tejidos blandos.
Títol de la investigació: Ganancia de volumen y estabilidad alrededor de implantes dentales después de la conexión del pilar utilizando injerto conectivo subepitelial de paladar o tuberosidad. Estudio clínico prospectivo aleatorizado.

Hem sol·licitat la seva participació en un estudi d'investigació. Abans de decidir si hi accepten participar, és important que comprenguin els motius pels quals es duu a terme la investigació: com s'utilitzarà la seva informació, en què consistirà l'estudi i els possibles beneficis, riscs i molèsties que pugui comportar.

En cas que participin en algun altre estudi, ho hauran de comunicar al responsable per a valorar si poden participar en aquest.

QUINS SÓN ELS ANTECEDENTS I L'OBJECTIU D'AQUEST ESTUDI?

L'objectiu principal d'aquest estudi es comparar el guany de geniva al voltant d'implants dentals utilitzan teixit de 2 zones diferents de la boca (paladar o tuberosidad). L'objectiu secundari es determinar quin dels dos teixits és més estable a llarg plaç.

En cada cas s'utilitzarà aleatoriament teixit d'una de les dues àrees posibles per aumentar la geniva al voltant del implant.

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Nombrosos estudis han descrit tècniques d'ingert de geniva per l'augment d'aquesta al voltant dels implants. Els resultats sugereixen que aquestes tècniques són beneficioses pels implants, ja que s'aconsegueix una geniva més forta a la zona, cosa que protegeix millor l'implant.

En aquest estudi es realitzaran escàners òptics i fotografies intraorals, els quals serviran per mesurar les variables del estudi. En cap cas aquestes imatges seran utilizades amb cap altre finalitat.

TINC L'OBLIGACIÓ DE PARTICIPAR-HI?

La decisió sobre participar o no en la investigació els correspon a vostès. En el cas de no voler participar o bé el volen abandonar, la qualitat de l'assistència que rebran no quedarà afectada. Si hi decideixen participar, els lliurarem el formulari de consentiment informat per tal que el signin.

En cas de no seguir les instruccions del doctor/a del estudi, o per qualsevol altre motiu justificat, s'optarà per sortir de l'estudi i realitzar el tractament habitual.

QUINES SÓN LES MEVES OBLIGACIONS?

Com a pacient haurà de ser conseqüent amb totes aquelles indicacions postquirúrgiques i en relació a la medicació a pendre-la per evitar complicacions. A més, s'haurà de comprometre a acudir a les visites de control postoperatori.

QUINS SÓN ELS POSSIBLES EFECTES SECUNDARIS, RISCS I MOLÈSTIES ASSOCIATS A LA PARTICIPACIÓ?

El pacient pot presentar les complicacions associades a qualsevol cirurgía oral, en les que s'inclou dolor moderat, sobreinfecció, edema, inflamació y hematomes eventualment.

QUINS SÓN ELS POSSIBLES BENEFICIS DE PARTICIPAR-HI?

El pacient aportarà un benefici inmediat en quant a la contribució en el coneixement i desenvolupament científic en l'àmbit de l'augment de geniva. A més, es beneficiarà del procediment d'augment de geniva, ja que demostrat en la literatura que aquest procediment és beneficioso pels implants dentals.

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COM S'UTILITZARAN LES MEVES DADES DE L'ESTUDI?

El tractament, la comunicació i la cessió de les dades de caràcter personal dels subjectes participants en l'assaig s'ajusten al que disposa la Llei orgànica 15/1999, de 13 de desembre, de protecció de dades de caràcter personal.

Aquestes dades, no inclouen ni el seu nom ni la seva adreça, sinó que se li assignarà un número de codi. Únicament l'equip investigador, tindrà accés a la clau del codi que permet associar les dades de l'estudi amb vostès. No obstant això, les autoritats reguladores, el comitè d'ètica independent o altres entitats de supervisió podran revisar les seves dades personals. L'objectiu de les revisions esmentades és garantir la direcció adequada de l'estudi o la qualitat de les dades de l'estudi.

Si en retiran el consentiment d'utilitzar les seves dades de l'estudi, no podran continuar participant en la investigació. Han de tenir en compte que els resultats de l'estudi poden aparèixer publicats en la bibliografia, si bé la seva identitat no serà revelada.

COM PUC ESTABLIR CONTACTE SI NECESSITO OBTENIR MÉS INFORMACIÓ O AJUDA?

Mitjançant la signatura d'aquest formulari, assenteixen que han estat informats de les característiques de l'estudi, han entès la informació i se'ls ha clarificat tots els seus dubtes.

En cas de patir un dany relacionat amb l'estudi o per obtenir resposta a qualsevol pregunta que pugui surgir durant la investigació, contactin amb:

Dra./Dr._____

Universitat Internacional de Catalunya

Adreça: C/ Josep Trueta, s/n, 08195, Sant Cugat del Vallès

Nº de telèfon: 93 504 20 00

Annex 4 :Informed consent

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CONSENTIMENT INFORMAT (TESIS DOCTORAL)

Codi de l'estudi: PER-ECL-2011-10-NF
Versió del protocol: 1.2
Data de la versió: : 7 mayo 2012
Data de presentació: 20 octubre 2014
Títol: Ganancia de volumen y estabilidad alrededor de implantes dentales después de la conexión del pilar utilizando injerto conectivo subepitelial de paladar o tuberosidad. Estudio clínico prospectivo aleatorizado.

Director/a de la Tesis: Dr. José Nart Molina.

Doctorant: Ernest Rojo Xicart.

Tutor/a / Monitor/a: -.

Departament: Periodoncia

Línia d'investigació: Aumento y estabilidad de volumen en tejidos blandos.

Títol de la investigació: Ganancia de volumen y estabilidad alrededor de implantes dentales después de la conexión del pilar utilizando injerto conectivo subepitelial de paladar o tuberosidad. Estudio clínico prospectivo aleatorizado.

Jo, el Sr./la Sra.:.....

- He rebut informació verbal sobre l'estudi i he llegit la informació escrita que s'hi adjunta, de la qual he rebut una còpia.
- He comprès el que se m'ha explicat.
- He pogut comentar l'estudi i fer preguntes al professional responsable.
- Dóno el meu consentiment per prendre part en l'estudi i assumeixo que la meva participació és totalment voluntària.
- Entenc que em podré retirar en qualsevol moment.

Mitjançant la signatura d'aquest formulari de consentiment informat, dóno el meu consentiment perquè les meves dades personals es puguin utilitzar com s'ha descrit en aquest formulari de consentiment, que s'ajusta al que disposa la Llei orgànica 15/1999, de 13 de desembre, de protecció de dades de caràcter personal.

Entenc que rebré una còpia d'aquest formulari de consentiment informat.

Signatura
Núm. de

Data de la signatura

Página **1** de **2**

Universitat Internacional de Catalunya

DECLARACIÓ DE L'INVESTIGADOR O LA INVESTIGADORA

La persona que signa aquest full de consentiment ha rebut, del professional, informació detallada de manera oral i escrita del procés i de la naturalesa d'aquest estudi d'investigació, i ha tingut l'oportunitat de preguntar qualsevol dubte pel que fa a la naturalesa, els riscs i els avantatges de la seva participació en aquest estudi.

_____ _____

Signatura Data de la signatura

Nom:

Annex 5: Consort checklist

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	130
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	19-36
	2b	Specific objectives or hypotheses	37-43
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	47
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	48
	4b	Settings and locations where the data were collected	49, 55, 56, 58
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	56-59
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	49-56
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	47
	7b	When applicable, explanation of any interim analyses and stopping guidelines	No
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	49
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	49
Allocation	9	Mechanism used to implement the random allocation sequence (such as sequentially	49

concealment mechanism		numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions.	48, 49
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	49, 55, 58
	11b	If relevant, description of the similarity of interventions	56
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	59
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	59
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	63
	13b	For each group, losses and exclusions after randomisation, together with reasons	63
Recruitment	14a	Dates defining the periods of recruitment and follow-up	48
	14b	Why the trial ended or was stopped	48
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	63
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	63-64
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	64-74
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	65-67, 74
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	64
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	90
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	77, 81, 83
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	77-89
Other information			
Registration	23	Registration number and name of trial registry	NCT03090906 /

			clinicaltrials.gov
Protocol	24	Where the full trial protocol can be accessed, if available	Parlty, clinicaltrials.gov
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Osteology Foundation – Young Researcher Grant

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Annex 6: Presentations and awards

These investigation obtained a grant and has been presented-awarded in national and international symposium:

- Osteology Spain Barcelona 2017. “Soft tissue volume gain around dental implants using autogenous subepithelial connective tissue graft from the palate or tuberosity. A randomized prospective clinical study”. *Rojo E., Stroppa G., Sanz-Martin I., Gonzalez-Martín O., Nart J.*

- First award at Osteology Research Forum – Clinical Research, at International Symposium Osteology Monaco 2016. Examining committee: Dr. Frank Schwarz, Dr. Michael Bornstein, Dr. William V. Giannobile. “Soft tissue volume gain around dental implants using autogenous subepithelial connective tissue graft from the palate or tuberosity - preliminary results of a randomised prospective clinical study”. *Rojo E., Stroppa G., Sanz-Martin I., Gonzalez-Martín O., Nart J.*

- Winner of the Osteology Young Researcher Grant 2015. Osteology Foundation, Lucerne, Switzerland.

- 48ª SEPA Reunión Anual Valladolid 2014. Research oral communication: Comparación de la Ganancia de Volumen alrededor de Implantes Dentales mediante Injerto de Tejido Conectivo obtenido del Paladar o Tuberosidad. Ensayo Clínico Controlado Aleatorizado. Resultados preliminares.” *Rojo E., Sanz-Martin I., Vallés C., Pascual A., Nart J.*

- XIV Curso de Metodología de Investigación en Periodoncia y Osteointegración SEPA 2013: “Finalist award PREMIO DENTAID at the best investigation protocol”. Project: “Ganancia de volumen y estabilidad alrededor de implantes dentales después de la conexión del pilar utilizando

injerto conectivo subepitelial de paladar o tuberosidad. Estudio clínico prospectivo.” *Rojo E., Sanz-Martin I., Gonzalez-Martín O., Nart J.*

SUMMARY

11. SUMMARY

The aim of the present study is to evaluate and to compare the volume gain around dental implants when a subepithelial connective tissue graft (SCTG) from palate or tuberosity is used. The most used donor area for soft tissue augmentation has been the autogenous connective tissue from the palate. However recent studies have showed that tuberosity tissue may possess better tissue qualities for soft tissue volume augmentation.

It has been shown that tuberosity connective tissue is denser with less fat and glandular tissue. Therefore, it could be speculated that this firmer tissue will have less shrinkage and will achieve more soft tissue gain.

In this randomized clinical trial 32 patients with 36 implants with localized volume deficiency have been included and received a SCTG from palate or tuberosity. Measurements using an intraoral optical scan have been done at baseline and 3 months. Also 20 samples were obtained at baseline for immunohistochemistry and descriptive histological analysis.

In conclusion both groups obtained volume gain at 3 months. No statistical significant differences were found, patients receiving palate SCTG obtained a median gain of 0,59 (0,35-0,81) mm compared with 0,75 (0,57-0,97) mm for the tuberosity group.